RELATIVE TO EGG BIOLOGICAL VALUES AND REPLACEMENT VALUES OF SOME CEREAL PROTEINS IN HUMAN SUBJECTS [1]

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This study on a group of 11 young men was performed in August and September, 1943. Four cereal breakfast foods — two of them whole oat products, one three-quarters oats, and another whole wheat — were tested on these subjects alternately with a whole egg diet, and the biological and replacement values of the contained protein calculated from nitrogen balances against similar balances from whole egg protein.

The whole oat proteins will be designated no. 1 and no. 2. The first [2] is sold in 5-lb. paper bags to institutions, like hospitals, hotels and restaurants, the second [3] in 1-lb. packages through the retail trade. The third product,[4] which was new in 1943, consisted of "75% ground oatmeal, 20% corn and rye flours, and the remaining 5% made up of salt, sugar, sodium phosphate, calcium carbonate, vegetable oil, iron salts, niacin, thiamin and riboflavin." We understand that in the course of its preparation the mixture is cooked to softness and squeezed through presses to make little doughnut-shaped rings, which are then dried and puffed. This latter process is carried out by subjecting the product "to a pressure of

[1] This work was supported by a grant from the Quaker Oats Company of Chicago.

[2] "Buckeye" Rolled Oats, made by the Quaker Oats Company.

[3] "H-O," manufactured by the Hecker Products Company.

[4] "Cheeriots," now called "Cheerios," manufactured by General Mills, Inc.

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80 to 100 lbs. at a temperature of 190°C. to 232°C. for 5½ to 6½ minutes," then letting off the pressure to produce the effect of popping corn. The starch granules are disrupted and are thus more easily attacked by the digestive juices. This product is here called the "puffed mixed cereal."

The 4th product,[5] made from whole wheat, is processed as follows: "It is 'scoured' to remove beard, fine dust and loose particles of the outer bran, then is steam cooked to 118°C., after addition of cane sugar (7%), salt (3%), and malt syrup (0.5%)." It is then dried and flaked (hence it is here called "flaked wheat") by passage between warm rollers and finally toasted "in hot-air ovens where the temperature of the hot air is varied along the line but at no point exceeding 180°C." The temperature of the product is, of course, less than that of the hot air. This last-named cereal was used in a former study (Murlin, Nasset and Marsh, '38) and a considerable reduction in the biological value of the protein as compared with that of other whole wheat products was obtained. We were interested to see if the values obtained 6 years earlier could be duplicated, or whether some improvement had been made to conserve the biological value of the protein. Stewart, Hensley and Peters ('43) have shown that the capacity to maintain growth in rats is definitely impaired by subjecting a mixture of oats, corn and rye (presumably identical with the product we have called "puffed mixed cereal") to the explosion or puffing process, as compared with the growth obtained on a similar mixture of the same cereals not so treated.

METHOD

The egg replacement method originally described by Sumner, Pierce and Murlin ('38) was adopted because it provides a diet which can be tolerated much longer and with far less alimentary distress by human subjects than the no-protein control diet employed in the more rigid biological value method of Thomas ('09) and of Martin and Robison ('24). A biological value of equal validity for comparative purposes

[5] "Wheaties," also manufactured by General Mills, Inc.

with that of these authors can be calculated on the assumption that the egg standard diet is attended by no greater alimentary losses of nitrogen than an extremely low protein diet,[6] or one containing no protein at all.

As in previous work from this laboratory, the egg standard diet and the experimental test diet each contained approximately 80% of the protein from egg or cereal, 10% from "4X" cream and butter, and 10% from all the remaining constituents: viz., a plain salad of lettuce and ripe tomato with seeds removed, a dish of applesauce of standard make, and orange marmalade or jam. The distribution of calories among the three proximate principles averaged 5% for protein, 50.1% for carbohydrate and 44.9% for fat. The ranges were from 4.5 to 5.4% for protein, 48.9 to 51.7% for carbohydrate, and 43.0 to 46.1% for fat (table 1). Calories per kilogram body weight per day averaged 45 and per square meter body surface (DuBois) 1,720 per day.

To make sure that each subject received sufficient vitamins throughout, a single polyvitamin tablet[7] was given to each member of the squad once a day, and for a mineral supplement two no. 0 capsules containing tricalcium phosphate and ferric phosphate were taken at each meal. These 6 capsules supplied 0.6 gm calcium, 0.3 gm phosphorus and 12 mg iron daily, in addition to the amounts contained in the food.

As the principal source of energy of the egg diet, a cornstarch muffin was baked of such a size that each provided approximately 207 Cal. The composition of this muffin — an evolutionary improvement on a previously used cornstarch "bread" (Sumner and Murlin, '38) — in an amount making 4 muffins, an average daily serving for one man, was as fol-

[6] For example, in the 1938 study (Murlin, Nasset and Marsh) referred to, there were 6 periods comprising a total of 30 days on the standard egg diet when the squad of 10 men showed an average daily excretion of only 0.77 gm fecal nitrogen. The diet contained an average total of 5.97 gm N. In a recent study (Hawley, Murlin, Nasset and Szymanski, '48) the average daily fecal excretion by 10 men on a protein diet for 7 days was 0.72 gm N. The diet contained only 0.290 gm N, aside from caffein N amounting to 0.117 gm.

[7] Upjohn's "Unicap."

lows in grams: Cornstarch 100, crisco 27, baking powder 5, "4X" cream 33, salt 2, egg 33, sugar 10, water 46, and gum arabic 20. It was baked in muffin tins to a light brown color and proved superior in flavor as well as in texture to the formerly used "bread" or "biscuit."

The "4X" cream was obtained through priority order by the Office of Price Administration in Washington from a reliable local source. The eggs were delivered fresh each day

TABLE 1

The diet squad

SUBJECT		AGE	HT.	SURFACE AREA	WEIGHT		CALORIES			
					Change Begin. to end		Average			
							Total	% distribution		
								P	CHO	F
		yrs.	*cm*	m^2	*kg*	*kg*		%	%	%
1	J.T.	25	169.7	1.75	65.9	— 0.5	3015	5	50.1	44.9
2	N.M.	29	178.0	1.91	74.4	+ 0.6	3338	5	48.9	46.1
3	R.B.	31	181	1.93	73.3	— 2.5	2907	5.4	51.6	43.0
4	J.S.	25	166.0	1.90	82.9	— 1.1	2892	5.1	50.4	44.5
5	N.T.M.	17	179	2.03	88.5	— 4.2	3637	5.0	49.7	45.3
6	R.G.	29	167.5	1.70	62.4	+ 0.6	2605	5.1	49.8	45.1
7	H.S.	21	179	1.90	72.4	— 0.8	3185	5.1	49.7	45.2
8	S.H.	18	179	1.83	66.5	— 2.3	3635	4.5	51.7	43.8
9	R.E.	18	169	1.71	63.8	— 2.1	3335	4.6	49.7	45.7
10	P.B.	30	189	1.87	64.4	+ 1.3	3064	5.0	50.0	45.0
11	L.E.	17	177	1.80	65.8	— 0.5	3435	4.7	50.0	45.3
	Av.	24.7	175.6	1.85	70.9	— 1.0	3185	5.0	50.1	44.9

from a farm which regularly supplies the Strong Memorial Hospital and all other items of the diet were of standard high quality, though (because of market limitations during the war emergency) not always from the same source.[8] Because the experiment was conducted through the months of August and September, few of the squad preferred hot drinks with all their meals. A few took coffee regularly for breakfast, and tea for lunch or supper. A popular carbonated beverage[9] was

[8] Oranges were always of the "Sunkist" brand, lettuce the "Iceberg" variety, tomatoes of the "Beefheart" variety when possible, and so forth. The orange marmalade was supplied by the W. N. Clark Company, Rochester, N Y.

[9] Coca-Cola.

permitted as a cooling drink as desired, in amounts up to two or three bottles daily. The nitrogen of the tea, coffee and carbonated beverage was regarded as completely excreted by the kidneys each day.

Aliquot samples of all foods were taken daily, and at the end of each 6-day period they were analyzed for nitrogen.

The diet squad

For a result based on figures obtained with 10 persons it has been found advisable in diet work of this character to start with at least 11 experimental subjects. Some one person is likely to make a serious error in collection or to lose appetite because of the monotonous diet in one or more periods and, as occasionally happens in hot weather even with the utmost precautions, a mild food infection may occur, producing diarrhea. This happened in period IV of the present experiment, causing loss of stools by two subjects. In this instance it was possible to substitute the mean fecal N of the remaining 9 men without throwing the final results askew.

The squad, all males, was composed of three instructors, one graduate assistant, three medical students not qualified for the Army or Navy program, and 4 college freshmen enrolled as pre-medical students. Their physical characteristics and the average distribution of calories for each are given in table 1. All of these men were intellectually interested in the results and were in need of financial reward for close adherence to the dietary regime and quantitative collection of excreta. Before the end, one of the medical students was obliged to withdraw because of persistent alimentary distress occasioned by the large proportion of carbohydrate contained in the diet, and one of the freshmen for a valid reason was excused from the last period on the egg diet. The total squad was divided into two groups, a and b, which ate the several test proteins in reverse order (table 2). The purpose of this arrangement was to counteract any advantage which might accrue to one cereal food as against another by the accident of position in the series. For example, it requires at least 10 days on a low

TABLE 2

Order of the periods and number of subjects in each group (in parentheses)
(All figures are reduced to daily values)

GROUP A					GROUP B				
Mean values for:					Mean values for:				
Urine N	Fecal N	Total excr. N	Diet N	N bal.	Urine N	Fecal N	Total excr. N	Diet N	N bal.
gm	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*
		I Egg (6)					I Egg (5)		
5.10	1.27	6.37	6.24	— 0.13	4.98	1.51	6.49	6.25	— 0.24
		II Oats 1 (6)					II Puffed mixed cereal (5)		
5.18	1.50	6.68	5.72	— 0.96	5.85	1.83	7.68	6.41	— 1.27
		III Egg					III Egg (5)		
(6) 4.86	1.16	6.02	6.65	+ 0.63	4.52	1.28	5.80	6.50	+ 0.70
(5) 4.68	1.17	5.85	6.73	+ 0.87					
		IV Flaked wheat (5)					IV Oats (5)		
(5) 5.90	1.96	7.86	6.45	— 1.41	5.13	1.56	6.69	6.35	— 0.34
		V Egg					V Egg (5)		
(5) 4.77	1.10	5.87	6.43	+ 0.56	4.07	1.33	5.40	6.37	+ 0.97
(6) 4.81	1.15	5.96	6.35	+ 0.39					
		VI Oats 2 (6)					VI Flaked wheat (5)		
5.57	1.47	7.04	6.42	— 0.62	5.68	1.91	7.59	6.42	— 1.17
		VII Egg					VII Egg		
(6) 4.42	1.04	5.46	6.47	+ 1.01	3.99	1.43	5.42	6.45	+ 1.03 (5)
(5) 4.38[1]	0.99	5.37	6.24	+ 0.87	4.33	1.40	5.73	6.91	+ 1.18 (4)
		VIII Puffed mixed cereal (5)					VIII Oats 1 (4)[2]		
5.12	1.81	6.93	6.15	— 0.78	5.82	1.55	7.37	6.70	— 0.67
		IX Egg (5)					IX Egg (4)		
4.39[1]	1.03	5.42	6.15	+ 0.73	4.12	1.63	5.75	6.80	+ 1.05

[1] Subject no. 5 ate egg in these two periods with group A but his egg periods belong to group B, period VIII.
[2] Subject no. 5 replaces no. 10 in this period and subject no. 9 is dropped because he had no egg period IX.

protein diet to reach a steady state in the rate of urinary excretion of nitrogen, because of the "deposit nitrogen" resulting from the previously relatively high protein diet. Therefore, as can be observed in table 2, which exhibits the average urinary N of all members in the successive egg periods, the average is 0.7 gm to 1.0 gm higher for the same number of subjects [10] in period I than in period VII, notwithstanding a somewhat higher ingestion of egg N in the later periods. This results in a higher N excretion in the first egg period than in any subsequent egg period, and in calculating the biological value the excess urinary nitrogen (cereal urinary N minus egg urinary N; see table 3) is less, as a percentage of the absorbed, in the first cereal period; consequently the biological value is higher than it should be. When, however, half of the squad eats the same cereal protein in the last test-protein period, the excess nitrogen is higher than it would be for them in the first cereal period, and consequently the biological value is lower. The advantage of the first position is offset by the disadvantage of the last. The gradual decline in average urinary N in successive egg periods is due to the fact that all cereal proteins have a lower B.V. than whole egg protein, and because of the accumulating deficit the egg protein is retained better as the series progresses.

RESULTS

a. Biological value

This measure of the nutritive value of a protein is defined briefly as the percentage of the absorbed N which is retained in the body. It differs from the egg replacement value in two ways: (a) It takes account of the relative alimentary losses on the standard protein and test protein, and, assuming that the former loss is minimal, it thus measures absorbed N by deducting the fecal food N for the test protein (obtained

[10] It will be observed that the average data for several egg periods had to be calculated for two different numbers of subjects, depending on the number who completed the adjacent cereal period successfully.

by the difference between fecal losses on the two proteins) from the fed N. In this study the assumption was thwarted in the case of one subject, who did not digest egg protein perfectly in *any* period, as was evident from obvious traces of it in the stools; (b) it takes account of metabolic excess N by deducting the urinary excretion of nitrogen on the egg protein diet from that on the cereal protein diet. All other nitrogenous constituents of the two diets being the same, this excess is a measure of the failure of the test protein to equal the egg protein in its value (supplemented by the other sources of nitrogen) to the tissues, as gauged by retention.

Theoretically, one should obtain biological values, and replacement values as well, by comparison of an intermediate test protein period with the preceding and following egg proteins. In this study, however, as in a preceding one on breads (Murlin et al., '41), it was noted that the average retention in each succeeding egg protein period was greater than in the one before. For this reason it was decided to employ the mean fecal and urinary nitrogen values from all the egg periods as the basis of calculation of biological values, instead of the data from the two periods adjacent to each test protein period. This was not done, however, before comparing one method with the other from the standpoint of concordance, or, in other words, determining which method resulted in the least variation among the several subjects as shown by the standard deviation. In fact, one other method was compared with these two by the same criterion, this method involving the use of the egg period immediately following the cereal period, thus eliminating the first egg period which, as noted above, gave clear evidence of a "hangover" effect from the previous higher protein diet. Neither of the two latter methods proved so satisfactory as the first, for their standard deviations were greater. The downward trend in the excretion of nitrogen from the first to the last egg period thus is compensated for not only by feeding the cereals in reverse order to the two groups of the squad (table 2), but also by averaging all the egg periods using a common plane

TABLE 3

Mean relative biological values for cereal proteins

	DATUM OF INTEREST	CEREAL			
		Oats no. 1	Oats no. 2	Puffed Mixed cereal	Flaked wheat
	No. of subjects	10	10	8	10
a.	Cereal fecal N (gm)	1.56	1.52	1.89	1.94
b.	Egg fecal N (gm)	1.20	1.27	1.27	1.28
c.	(a–b) Difference (gm)	0.36	0.25	0.62	0.66
d.	Total food N (gm)	6.07	6.38	6.34	6.44
e.	(d–c) Absorbed N (gm)	5.71	6.13	5.72	5.78
f.	True digestibility (%)	93.9 ± 3.3	96.1 ± 4.9	90.2 ± 3.5	89.7 ± 2.6
g.	Cereal urine N (gm)	5.21	5.28	5.57	5.78
h.	Egg urine N (gm)	4.63	4.54	4.58	4.44
i.	(g–h) Excess N (gm)	0.58	0.73	0.99	1.35
j.	(e–i) Retained N (gm)	5.13	5.40	4.73	4.43
k.	$\frac{j \times 100}{e}$ = Biol. val. (%)	89.9	88.1	82.8	76.7
l.	Mean deviation from mean biol. val. (gm)	± 3.0	± 3.9	± 4.4	± 4.5
m.	Standard deviation (gm)	3.81	5.56	6.52	5.85

Significance of differences between mean biological values:

(1) No. 1 Oats and puffed mixed cereal. n = 18, "t" = 6.0, P = .015.
(2) No. 1 Oats and flaked wheat. n = 18, "t" = 6.0, P = .0001.
(3) No. 2 Oats and flaked wheat. n = 18, "t" = 4.47, P = .0002.
(4) Flaked wheat and puffed mixed cereal. n = 16, "t" = 2.03, P = .06.

of reference. Each cereal food, therefore, occupies the same average distance from the midpoint sequentially, and is topographically oriented to the same fundamental datum.

In calculating biological values and the statistical significances between mean values for the different cereals it was necessary to exclude extreme variants in all the data for the 4 cereals — one each for the two oats cereals and flaked wheat, and two for the puffed mixed cereal. Only those which fulfilled the Chauvenet criterion for rejection of extreme variants in small populations (as this is set forth by Davenport and Ekas, '36) were discarded.

The significances of the differences between pairs of means, according to Fisher's method, are shown in the footnote to table 3. It is evident that the two whole oat products give higher biological values than the more extensively pretreated

ones, and that in all comparisons but one the differences between means are highly significant.

The true digestibilities of the several cereals compared with those of all the egg periods averaged together do not, however, exhibit differences of any significance. The differences become very significant when they relate to what happens after absorption. Observe by how much more the cereal nitrogens exceed the egg urine nitrogens in the last two columns of table 3 than in the first two. This table also shows how the differences are subtracted from the absorbed nitrogens to get the nitrogens retained (line j) and how the latter are much less for the last two columns, thereby giving lower biological values (line k).

The cereal fecal nitrogens in the last two columns exceed the basal egg fecal nitrogens by nearly twice the same difference in the first two (line c). This results in lowering the amount of absorbed nitrogen, and explains the lower digestibilities of the two cereal products represented. By the same token, the lower absorption should serve (other factors being equal) to raise the biological values, but the greater excess of urine nitrogen from the same two cereals means less retention, and that diminution more than offsets the effect on absorption.

b. Egg replacement values (E.R.V.)

As the term is used in this laboratory, the E.R.V. is calculated from the crude N balance data, as shown in table 4. It may be defined as the extent, percentage-wise, to which any particular protein can replace egg protein *in maintaining N balance*. Ideally, the egg and test protein should be fed at exactly the same levels and, as may be seen in table 4, this ideal was approximated very closely in tests with all but the first cereal listed. This particular failure was due to using a wrong value for the nitrogen in this cereal. An adjustment in the difference between N balances was therefore made, upon the reasoning that if a lower intake of cereal protein produced a given minus N balance, a higher intake

(equal to that of the egg period) would have produced a lesser minus N balance in inverse proportion to these levels of intake. Thus, in the present instance, for group a, 6.44: 5.72 :: 1.22 : x, and x = 1.08. This corrected difference in N balances weighted against a similarly adjusted difference in balances for group b gives the weighted final difference between balances, which determines the extent to which the cereal failed to replace egg protein.[11] This difference of balances, expressed as a percentage of the egg N ingested and subtracted from 100, is the extent to which the cereal protein *did* replace egg protein. A similar small adjustment was made for the results with a and b groups on all cereals where significant differences in N intake existed.

It is evident from table 4 that the two whole oat products excel in their ability to replace egg protein and in the maintenance of N balance, and that the two more extensively pretreated products — one from wheat and the other from a mixture of the three cereals, oats, corn and rye fall considerably below this level. In order to say exactly how much the wheat product was reduced in this capacity, a whole wheat in its natural form or with a minimum amount of pre-treatment should have been tested in parallel experiments with the same squad, as was done in our earlier study (Murlin et al., '38) and by Kuether and Myers ('48). However, it is of interest that we obtained a replacement value with this product only a few points above that found in 1938, which may be explained by the fact that the ingestion level of nitrogen averaged 0.6 gm higher than in the earlier study. No such combination of oats, cornmeal and rye in natural form was available for comparison with the puffed mixed cereal.

[11] Such an adjustment leaves something to be desired, of course, because failure of a given protein to maintain N-balance is due presumably to the critical level at which one or more of the essential amino acids fails to suffice for some governing protein synthesis or syntheses. We are not yet in possession of sufficient information regarding these critical levels to make adjustments on the basis of known amino acid compositions. Studies like those of Block and Mitchell ('46) show the direction of advance in these matters.

TABLE 4

Egg replacement values of cereal proteins

1	2	3	4			5	6	7		8	9	10	11	12	13	
		FOOD N					N BALANCES									
GROUPS	CEREAL FOOD	Cereal	Weighted mean of egg periods	Before and after periods		Difference	Cereal	Mean of egg periods before and after		Difference	ADJUSTED DIF.	NO. OF SUBJ.	WGT'D. FINAL AV. DIF.	% OF MEAN EGG N	% REPLACEMENT OF EGG N	ST. DEV.
a	Oats no. 1	5.72	6.60	6.44		−0.72	−0.96	+0.26		1.22	1.08	6	1.34	20.4	79.6	7.2
b		6.70		6.85		−0.15	−0.67	+1.11		1.78	1.74	4				
a	Oats no. 2	6.42	6.42	6.41		+0.01	−0.62	+0.69		1.31	1.31	6	1.24	19.3	80.7	8.3
b		6.35		6.43		−0.08	−0.33	+0.83		1.16	1.15	5				
a	Puffed mixed cereal	6.15	6.28	6.19		−0.04	−0.78	+0.80		1.58	1.57	5[1]	1.54	24.5	75.5	5.9
b		6.41		6.37		+0.04	−1.27	+0.23		1.50	1.51	5				
a	Flaked wheat	6.45	6.49	6.58		−0.13	−1.41	+0.71		2.12	2.08	5[2]	2.12	32.7	67.3	6.6
b		6.42		6.41		+0.01	−1.17	+1.00		2.17	2.17	5				

[1] Number 5 is excluded from this group as an extreme variant.

[2] Number 4 is excluded from this group as an extreme variant.

The greatest differences between means in the replacement values shown in column 13 of table 4 are those between the two whole oats products and the flaked wheat. The difference between the means of no. 2 oats and the puffed mixed cereal is just short of statistical signficance for 11 and 10 subjects, respectively.

DISCUSSION

The results of our experiments confirm the findings of Kuether and Myers ('48) for their several experiments on two products, which presumably are in all particulars the same as those used in this study. Rolled oats, in the experience of this laboratory, gives the same replacement value whatever its trade name. It is, however, possible that the product the above workers call "exploded oats" has undergone some change in formula or manufacturing process from the one called "puffed mixed cereal" used in this laboratory 5 years earlier.

It is puzzling to the present authors why, in collection periods of 10 to 12 days with cereal as the sole source of nitrogen in the diet, Kuether and Myers did not obtain any closer agreement in nitrogen balances than has been obtained in this laboratory in periods of 6 days each on three cereals of identical nature supplemented by milk and fruit nitrogen. The impression is given in their table 6 of their having struck a N balance every single day for each subject, whereas from the description of their method it is clear that stools were separated only in periods of several days and that a single value for the period was obtained by dividing by the number of days. The variable in known excretion values from day to day, therefore, can only be the urine nitrogens. It is decidedly misleading to give daily variations in *balance* as "standard deviations" when they are really variations only in the urine nitrogen. The number of subjects referred to in their table 6 is only three, and there are obvious deviations among them that in sum amount at times to more than the mean value shown, so that the mean deviation from the mean is more than 50%

of the mean. This is true of the "exploded oat cereal" and the whole wheat, which gave the lowest mean nitrogen balances for the three subjects. The "standard deviations" given for each subject to indicate day-to-day variations, also are at times higher than the mean of "nitrogen balances." It would have been of interest, but of no more general application, if the authors had merely compared one subject with another.

The opinion expressed in a former paper (Murlin et al., '38) that wheat flakes exhibited a reduction in biological value due to heat treatment appears to be confirmed by the present results, for the value reported in table 3 is considerably lower (76.7 *cf.* 82.1) than that reported earlier, although the heat treatment appears to be less severe (see p. 406).

There is confirmation also of the reduced biological value for the growth of rats of the puffed mixed cereal reported by Stewart et al. ('43) in the low B.V. of this protein for maintenance in man as shown in table 3. There are no results in this communication confirming an earlier finding that undamaged oats are superior to undamaged wheat in biological value or replacement value. Kuether and Myers ('48), however, seem to stand alone in their findings of the reverse.

SUMMARY

Four cereal products prepared as "breakfast foods" have been studied with reference to the biological value for human subjects of the contained proteins based on egg protein as standard. Two of the products were whole oat preparations; one, three-quarters oats; and one, whole wheat with "all of the original nutrients retained."

The egg replacement method as previously employed in this laboratory was followed and some improvements were made. The squad was divided into two groups, which ate the cereals in reverse order, thereby counteracting any advantage from mere position in the series of 9 periods — 5 on egg, 4 on cereals.

The biological values of the cereal proteins were calculated for each individual on the basis of his average data from all

the egg periods. Hence, each cereal food occupied the same average distance from the nitrogenous midpoint of the series of standard diets.

After calculation of the biological values and the replacement values in terms of the egg standard, and obtaining standard deviations, the extreme variants for each cereal were eliminated in accordance with standard statistical procedure.

The biological values relative to egg protein of the 4 cereal products recalculated with their standard deviations for the number of subjects retained were found to be as follows: no. 1 oats 89.9 (3.81), no. 2 oats 88.1 (5.56), puffed (or "exploded") oats-corn-rye cereal 82.8 (6.52), and flaked wheat 76.7 (5.85). The differences between the means for the two oats products and wheat flakes are highly significant, as is also true for the no. 1 oats and the puffed oats-corn-rye cereal but not for the latter and wheat flakes.

The replacement value, defined as the extent to which any particular protein can replace egg protein in the maintenance of nitrogen balance, was calculated for each group of subjects for each cereal. The order of values derived from weighted differences in average N balances between cereal and egg are as follows: no. 1 oats 79.6 (7.2); no. 2 oats 80.7 (8.3); puffed oats-corn-rye mixture 75.5 (5.9), and wheat flakes 67.3 (6.6). Only the differences of means between the two whole oats products and wheat flakes are statistically significant.

LITERATURE CITED

BLOCK, R. J., AND H. H. MITCHELL 1946 The correlation of the amino acid composition of proteins with their nutritive value. Nutrition Abstr. and Rev., *16:* 249.

DAVENPORT, C. B., AND M. P. EKAS 1936 Statistical Methods in Biology, Medicine and Psychology. John Wiley and Sons, Inc., New York N. Y., p. 21.

HAWLEY, ESTELLE E., J. R. MURLIN, E. S. NASSETT AND T. A. SZYMANSKI 1948 Biological values of six partially-purified proteins. J. Nutrition, *36:* 153.

KUETHER, C. A., AND VICTOR C. MYERS 1948 The nutritive value of cereal proteins in human subjects. Ibid., *35:* 651.

MARTIN, C. J., AND R. ROBISON 1924 The minimum nitrogen expenditure of man and the biological value of various proteins for human nutrition. Biochem. J., *16:* 407.

MURLIN, J. R., MARGARET E. MARSHALL AND CHARLES D. KOCHAKIAN 1941 Digestibility and biological value of whole wheat breads as compared with white breads. J. Nutrition, *22:* 573.

MURLIN, J. R., E. S. NASSET AND M. E. MARSH 1938 The egg-replacement value of the proteins of cereal breakfast foods with a consideration of heat injury. Ibid., *16:* 249

STEWART, R. A., W. HENSLEY AND F. N. PETERS, JR. 1943 The nutritive value of protein. I. The effect of processing on oat protein. Ibid., *26:* 519.

SUMNER, EMMA E., H. B. PIERCE AND J. R. MURLIN 1938 The egg-replacement value of several proteins in human nutrition. Ibid., *16:* 37.

SUMNER, EMMA E., AND J. R. MURLIN 1938 The biological value of milk and egg protein in human subjects. Ibid., *16:* 141.

THOMAS, K. 1909 Über die biologische Wertigkeit der Stickstoffsubstanzen in verschiedenen Nährungsmitteln. Arch. Anat. u. Physiol., Physiol. Abth., p. 219.

IMPROVING THE NUTRITIVE VALUE OF FLOUR

III. THE USE OF ENRICHED AND NON-ENRICHED FLOUR IN DIETS SIMILAR TO THOSE CONSUMED BY CERTAIN LOW-INCOME GROUPS IN SOUTH CAROLINA [1]

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FOUR FIGURES

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Experiments have been reported (Westerman and Bayfield, '45; Westerman and Hall, '47; Guerrant and Fardig, '47; Westerman and Templeton, '48) in which enriched and non-enriched flour were used as the sole source of the B vitamins in the diets of experimental animals. In these experiments a fairly purified diet was used. The human dietary contains other foods besides cereals which furnish B vitamins; it seemed likely, therefore, that using diets made up of natural foods comparable to those consumed by human beings would give added information relating to this problem. The diets used in the experiments described in this report were patterned after those consumed by people with poor food habits or with low incomes. Such groups of individuals are most likely to include considerable quantities of cereals in their

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diets, either from habit or from economic necessity, since such foods represent cheaper sources of energy.

EXPERIMENTAL PROCEDURE

Albino rats weighing between 45 and 55 gm, and 28 days of age, were depleted of their body stores of B vitamins by placing them on a B-complex-free diet for 10 to 14 days. The animals were grouped in such a way as to have an equal distribution in regard to sex and litter mates. Six or more animals were placed on each diet.

The diets were patterned after those described by Moser ('45) as being consumed by some of the rural families in Pickens County, South Carolina. These data were calculated on the basis of daily consumption per individual, and the diets for the rats were based on the amount of each of the different foods consumed per person per day. In order to facilitate the preparation of the food, it was necessary to make some modifications in the diets and to group certain foods together. Carrots were used to represent yellow vegetables, and green beans, spinach, cabbage and broccoli were used alternately as the green vegetables. An average of the nutritive values of these vegetables was used in the calculations. In order to have a fair representation of the meat used in the average diet, beef and pork were mixed equally. Apples were used as representative of the fruits in the diet and dried beans and peas were the legumes. Dried whole milk was used in order to reduce the liquid content of the diet. The fats were margarine and salad oil. All cereals were added to the diets as enriched flour, non-enriched flour, ground whole wheat or cornstarch. The whole wheat was included in order to make a comparison of the diets having a whole grain cereal as against those containing enriched or non-enriched flour. A purified carbohydrate, cornstarch, replaced the cereal in some of the diets, in an attempt to discover by such replacement whether other constituents of the diets were furnishing enough B vitamins for adequate nutrition. The foods requiring cooking were cooked. The wet ingredients were mixed

and finely divided in a blender and then mixed with the dry ingredients in such a way that the diets were homogeneous. They were prepared once each week and kept in frozen storage until needed.

Calculations of the nutritive values of the different diets were made by using an average of the values by Sherman ('46) and Turner ('46), except for the flour and wheat; these were assayed. The enriched flour contained 6.94, 3.00 and 1.15 μg per gram of thiamine, riboflavin and pantothenic acid, respectively; the non-enriched flour 1.35, 0.74 and 1.12 μg per gram; and the whole wheat 5.15, 1.06 and 3.19 μg per gram of these same vitamins.

Four different experiments were conducted using cereals to furnish 62, 50, 40 and 33% of the total calories in the diets. It might seem that 62% of the calories from cereals is a rather large amount and it may be that people in the United States do not as a rule consume cereals in this amount, but there are countries in Europe where cereals are the main source of calories. Moser ('45) has shown that some of the people in South Carolina do consume cereals at levels of 40 and 50% of the total calories, and cereals may furnish 30% of the calories in other American diets. The constituents of the diets and the percentage of calories supplied by each are shown in table 1. The growth studies were carried out over periods of 9 to 14 weeks, and in some cases through the second generation.

RESULTS AND DISCUSSION

First experiment: Cereals furnished 62% of the total calories

In experiment I the cereals in the diets were as follows: diet I contained enriched flour, diet II non-enriched flour, diet III ground whole wheat and diet IV cornstarch. Growth studies were conducted over a period of 9 weeks and figure 1 gives the average weekly weight gains made by the animals on the different diets. The rats on diet I with enriched flour furnishing 62% of the total calories in the diet gained on the

average 17 gm more than those on diet II with non-enriched flour. Those on diet III with whole wheat gained 6 gm more than those on the diet with the enriched flour. The animals on diet IV containing cornstarch made the least gains during the 9-week period.

A comparison of the nutritive values of the 4 diets is shown in table 2. The nutritive values of all the diets except the one containing cornstarch were well within the range of those established as adequate for the normal person. The

TABLE 1

Composition of diets based on the daily food consumption of humans

FOOD	EXPERIMENT I		EXPERIMENT II		EXPERIMENT III		EXPERIMENT IV	
	Calories	Cal.	Calories	Cal.	Calories	Cal.	Calories	Cal.
		%		%		%		%
Cereal	2396	62.0	1200	40.0	888	32.8	1122	49.9
Sugar	143	3.7	661	22.0	398	14.7	131	5.8
Fat	432	11.2	220	7.2	432	15.9	388	17.2
Meat	142	3.7	114	3.8	142	5.2	142	6.3
Milk	372	9.6	298	9.9	372	13.7	166	7.4
Eggs	24	0.6	79	2.6	24	0.9	21	0.9
Legumes	158	4.1	140	4.7	98	3.6	42	1.9
Potatoes	58	1.3	150	5.0	213	7.8	131	5.8
Carrots	45	1.2	40	1.2	45	1.7	22	1.0
Apples	64	1.7	63	2.1	64	2.4	61	2.7
Green vegetables	33	0.9	31	1.0	33	1.2	25	1.1

diets containing enriched flour and whole wheat were considerably higher in the B vitamins and iron than the other two diets. This probably accounts for the greater average weight gains of the rats on these diets. The low protein content of diet IV containing cornstarch, as well as the smaller quantities of the B vitamins, undoubtedly were responsible for the slow growth of these animals. The protein deficiency was corrected in the experiments to follow by including vitamin-free casein in the diet along with the cornstarch. The results of this experiment would seem to indicate that enriched flour is superior to non-enriched flour in supporting

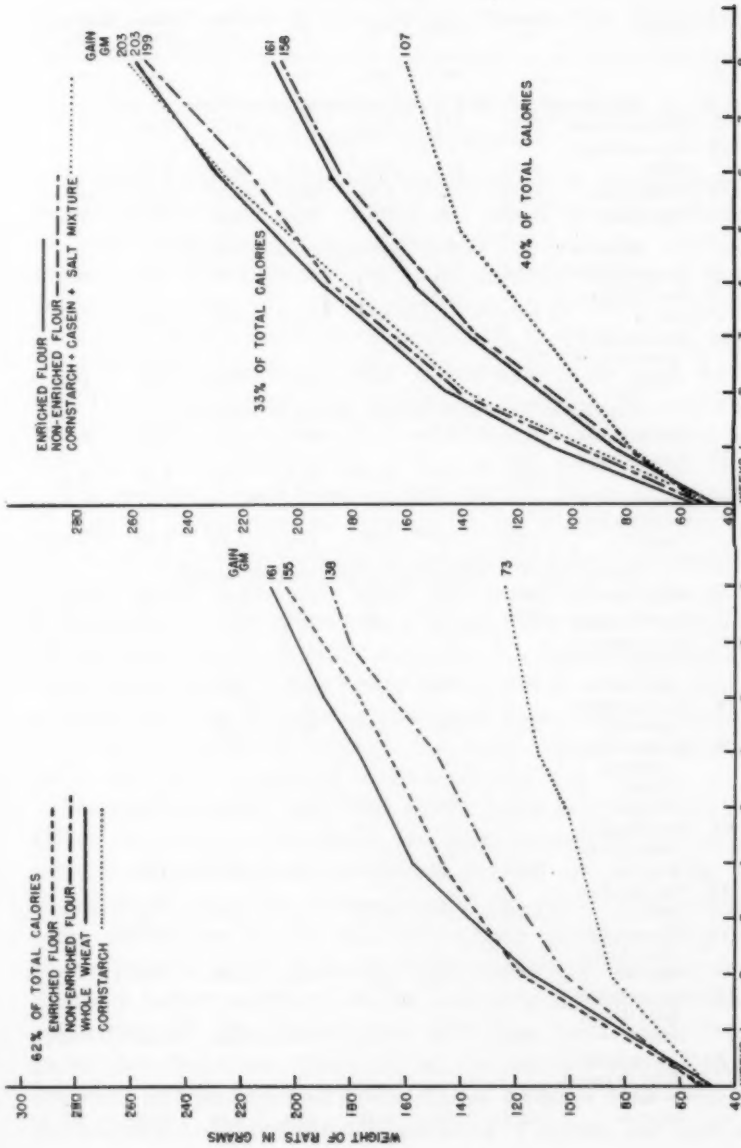

Fig. 1 Growth curves of animals in experiment I.
Fig. 2 Growth curves of animals in experiment II and experiment III.

TABLE 2

Nutritive value of daily diets as consumed by humans

	DIET	Calories	Prot.	Ca	Fe	Thiamine	Riboflavin	Nicotinic acid
			gm	*gm*	*mg*	*mg*	*mg*	*mg*
	Experiment I: 62% of total calories from cereals							
I	Enriched flour	3867	118	1.11	29.6	5.75	3.69	29.3
II	Non-enriched flour	3867	118	1.11	14.7	2.04	2.13	11.1
III	Whole wheat	3908	127	1.26	36.3	4.74	2.47	43.5
IV	Cornstarch	4171	45	0.93	10.0	1.11	1.67	5.7
	Experiment II: 40% of total calories from cereals							
I	Enriched flour	2996	81	0.86	20.0	2.61	2.48	18.6
II	Non-enriched flour	2996	81	0.86	13.0	1.36	1.71	9.5
III	Cornstarch + vitamin-free casein	3148	85	0.80	11.0	1.09	1.57	6.8
	Experiment III: 33% of total calories from cereals							
I	Enriched flour	2709	67	0.97	17.0	2.32	2.36	16.6
II	Non-enriched flour	2709	67	0.97	11.0	1.29	1.78	9.8
III	Cornstarch + vitamin-free casein	2722	67	0.93	10.0	1.19	1.68	7.8
	Experiment IV: 50% of total calories from cereals							
I	Whole wheat	2270	66	0.62	18.1	2.51	1.28	23.4
II	Enriched flour	2251	62	0.52	15.1	2.98	1.85	16.8
III	Enriched flour + salt mixture	2251	62	1.15	31.4	2.98	1.85	16.8
IV	Enriched flour + casein	2371	92	0.52	15.1	2.98	1.85	16.8
V	Enriched flour + casein + salt mixture	2371	92	1.15	31.4	2.98	1.85	16.8
VI	Cornstarch + casein + salt mixture	2513	58	1.10	22.2	0.80	0.90	5.7
VII	Non-enriched flour	2251	62	0.52	8.1	1.24	1.12	8.2
VIII	Non-enriched flour + salt mixture	2251	62	1.15	24.4	1.24	1.12	8.2
IX	Non-enriched flour + casein	2371	92	0.52	8.1	1.24	1.12	8.2
X	Non-enriched flour + casein + salt mixture	2371	92	1.15	24.4	1.24	1.12	8.2

growth in rats when these materials furnish 62% of the total calories of the diet.

Second and third experiments: Cereals furnished 40 and 33% of the total calories

In these experiments it was decided to lower the amount of cereal in the diets and to test the effect of the inclusion of more milk, fruit, and vegetables. The calories obtained from sugar were increased in experiment II and those from fat in experiment III. The composition of the diets is shown in table 1 and the nutritive value in table 2. Three diets were used in each experiment: diet I, containing enriched flour; diet II, containing non-enriched flour; and diet III, containing cornstarch with vitamin-free casein added to bring the protein level of this diet up to that of the other diets.

The results of the 8-week growth test are shown in figure 2. It may be noted that the animals on the diets with 40% of the calories from enriched and non-enriched flour maintained the same growth rate, but those with cornstarch at the same level gained on the average much less. The nutritive values of the diets (table 2) show that diet I, containing enriched flour, had a higher content of iron and the B vitamins, but it appears that diet II, with non-enriched flour, supplied enough of these materials to support the same amount of growth while diet III, containing cornstarch, did not.

In experiment III with 33% of the total calories from enriched and non-enriched flour, and from cornstarch with vitamin-free casein added, the growth rate of the rats (fig. 2) on all three diets was approximately the same. There were some differences in the nutritive values of these diets as shown in table 2, but evidently the materials in the diets furnished a better source of all the nutrients than did those in experiment II. The rats on these diets also made much better gains than did those with 40% of the calories coming from enriched or non-enriched flour. These animals with 33% of the calories from cereals also obtained a larger per cent of

calories from fat, meat, milk, and potatoes than those at the 40% level, and this may account for the better growth, as fat would have a sparing action on carbohydrate metabolism and meat and milk would furnish more of the B vitamins. These results indicate that when meat, milk, and vegetables are present in adequate quantities in the diet and there is less consumption of cereals, the benefits from the use of enriched flour are not easily demonstrated. This was also shown by Winters ('47), who fed rats on diets representative of the average American diet with 30, 33, and 36% of the total calories derived from cereals. No significant differences in the growth of the animals fed the enriched and non-enriched cereals at these levels were noted.

Fourth experiment: Cereals furnished 50% of the total calories

The data on food consumption of low-income groups as given by Moser ('45) did not allow for any waste of food. It was expected, however, that there would be some waste in preparing the fruits and vegetables and that all the food placed upon the table would not be eaten. It was decided that at least 10% of the estimated amount of food taken into the homes would be wasted as far as human consumption was concerned. Therefore the food consumption was recalculated for the purpose of the present study on the basis of a 10% loss. The milk was reduced from 60 to 30 gm. While a rural population may have more milk in the diet, it was thought urban families on low incomes would have about one-half as much.

With the above modifications, the cereal component of the diets furnished about 50% of the total calories. In this experiment some diets with vitamin-free casein and salt mixture added were included, so that protein and minerals would not be deficient. The vitamin-free casein provided 5.1% of the total calories and the salt mixture made up 0.5% by weight of the total diet. The constituents of the diets and the percentage of calories supplied by each are given in table 1, and the nutritive values in table 2. It may be noted that the

protein content of the diets with added casein is about one-third greater than that of the other diets. The calcium content of the diets without the added salt mixture was inclined to be low, due in part to the small amount of milk, which approximated one cup per day per individual. The iron content of diets VII and IX was low due to the use of non-enriched flour. Diets VI through X, containing cornstarch and non-enriched flour, were much lower in the B vitamins than diets I through V which contained the enriched flour.

The average weekly weight gains of the animals on the different diets are shown in figure 3. For purposes of comparison the weights of the animals with enriched flour in the diet were grouped with those on the corresponding diet containing non-enriched flour. As is shown by the growth curves in figure 3, the diets containing the enriched flour and the whole wheat supported about the same rate of growth in the rats, as both these groups made an average weight gain of 239 gm during the 13-week period. Those animals with non-enriched flour in the diet grew at a slower rate and gained a total of 229 gm during the same period. Those with cornstarch replacing cereal in the diet showed the least gain, 186 gm, even though this diet contained vitamin-free casein and salt mixture. It would seem that the B vitamins contributed by the enriched flour played an important part in the growth of the rats, and were factors in promoting slightly better growth in animals having enriched flour in the diet as compared with those on the diet containing non-enriched flour.

When salt mixture was added to the diets containing enriched and non-enriched flour, the weight gains were 20 gm more than without the added salt mixture, but there was an 11-gm difference in the gains made when enriched flour was in the diet over those made on non-enriched flour. When vitamin-free casein was added to increase the protein content of the diet, the growth rate was increased on both diets but the rats on enriched flour made slightly better gains.

When both salt mixture and protein were added, the gains were even greater but there was some difference between the

weight gains of the animals on the enriched flour diet over those rats on the non-enriched flour. It is likely that if more milk were included the diet containing non-enriched flour would be more nearly adequate in minerals and proteins as well as the B vitamins.

The results of the growth test indicate that enriched flour aids in promoting growth when milk and vegetable consumption is lowered and cereal consumption increased. The addition of the mineral salts and protein to the diet was beneficial, but slightly better growth rates were obtained with the use of enriched flour in either of these diets. It seems likely that the rat, being a fast growing animal, requires large quantities of minerals and proteins.

At the end of 13 weeks on the test diets the males and females were allowed to breed. The females on the diet containing enriched flour gave birth to more young than did those with non-enriched flour in the diet. For some unexplained reason none of the young of the mothers on diets II and VII lived. The females on diet III containing enriched flour and salt mixture, and those on diet VI containing cornstarch, salt mixture and casein, raised 15 young; however, the young of rats on diet III had an average weight of 55 gm at 28 days of age while those of animals on diet VI weighed 38 gm. A comparison of the young of rats on diet V, containing enriched flour, salt mixture, and casein, with those of animals on a similar diet containing non-enriched flour shows approximately the same number of young raised, but those of females with enriched flour in the diet weighed on the average 12 gm more than those of rats on the other diet. These data indicate that enriching the flour aids in reproduction and in the raising of normal young.

When the young rats of the second generation were 28 days old they were divided into groups of 6 with equal distribution as to sex and litter mates, and kept on some of the same diets. Since the females on the diets containing enriched and non-enriched flour did not raise their young, it was necessary to transfer rats from the other groups to these diets. The

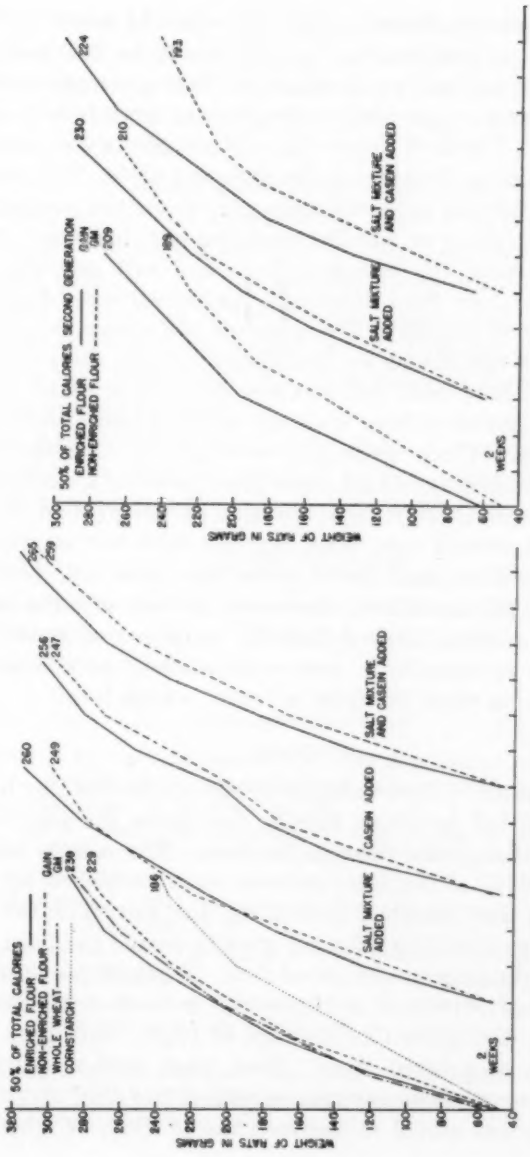

Fig. 3 Growth curves of first generation animals in experiment IV.

Fig. 4 Growth curves of second generation animals in experiment IV.

growth test was conducted over a period of 14 weeks so that, for purposes of comparison, the rats would be the same age at the end of the test as those of the first generation which had been placed on a depletion diet for one week before starting the test. Figure 4 shows the differences in the average total gains among the rats on the different diets. The rats on the diet containing enriched flour gained on the average 20 gm more than those on the diet with non-enriched flour; those on the diet with the enriched flour and salt mixture also gained 20 gm more than those with the non-enriched flour and salt mixture in the diet. The greatest difference in average weight gains was shown by the animals on the diets containing enriched flour with salt mixture and vitamin-free casein, and the non-enriched flour with salt mixture and vitamin-free casein added. Those animals receiving the enriched flour gained on the average 31 gm more than those on a similar diet with non-enriched flour. The addition of the protein did not improve the growth rate, since the rats with salt mixture included in the diets made better gains than those with both salt mixture and protein added. However, the test with the second generation animals showed that for purposes of growth enriched flour is superior to non-enriched flour as a source of the B vitamins when the flour is fed at a high level.

SUMMARY

Combinations of foods similar to those consumed by human beings were fed to albino rats to determine the significance of the use of enriched flour in the diets. The results indicate that when 62% of the total calories were furnished by flour, the animals with enriched flour in the diet gained on the average 17 gm more during a 9-week growth period than did those on diets containing non-enriched flour. When 50% of the total calories were furnished by flour, the animals with enriched flour in the diet gained an average of 10 gm more than those receiving non-enriched flour. Even when salt mixture was added to increase the calcium content of the diet, or vitamin-free casein was added to increase the protein, or when both

of these were added to the same diets, the rats receiving the enriched flour made the greater gains. The second generation animals showed a wider variation, as those fed enriched flour gained on the average 20 gm more than those with non-enriched flour in the diet.

The average weight gains made by the animals with 33 and 40% flour in the diet, respectively, showed no differences between those receiving enriched and non-enriched flour. The animals with 33% of the calories from enriched and non-enriched flour made better weight gains than those whose diets included flour at the 40% level. This was due probably to the added milk, meat, and vegetables in the former diet, which replaced the cereals.

The results indicate that when flour is consumed in large quantities the enriched product aids in promoting a better growth rate in rats than the non-enriched form.

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LITERATURE CITED

GUERRANT, N. B., AND O. B. FARDIG 1947 The thiamine and riboflavin content of whole wheat, non-enriched and enriched flours and breads made therefrom. J. Nutrition, *34:* 523.

MOSER, ADA M. 1945 The food supply of rural families in the six mile area of Pickens County, 1939–40 and 1942–43. S. Carolina Agr. Expt. Sta. Bull. 360.

SHERMAN, HENRY C. 1946 Chemistry of Food and Nutrition. The Macmillan Co., New York, N. Y.

TURNER, DOROTHEA 1946 Handbook of Diet Therapy. University of Chicago Press, Chicago, Ill.

WESTERMAN, B. D., AND E. G. BAYFIELD 1945 Enriched, Morris type and whole wheat flour as sources of the B-complex vitamins. J. Nutrition, *29:* 27.

WESTERMAN, B.D., AND GERTRUDE HALL 1947 Improving the nutritive value of flour. I. The effect of supplementing enriched flour with other B-complex vitamins. Ibid, *33:* 301.

WESTERMAN, B. D., AND FRANCES TEMPLETON 1948 Improving the nutritive value of flour. II. Further studies on the effect of supplementing enriched flour with B-complex vitamins and some observations on the use of 80% extraction flour. Ibid, *36:* 187.

WINTERS, JET. C. 1947 Some Trials of Human Diets on Lower Animals. Appraisal of Human Dietaries by Animal Experiment. Williams-Waterman Fund, New York, N. Y.

RIBOFLAVIN METABOLISM OF YOUNG WOMEN ON SELF-SELECTED DIETS

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Relatively few riboflavin metabolism studies using young women as subjects have been made. So far as is known, none has been made solely with subjects on a self-chosen diet, although several have observed urinary excretions. The 24-hour urinary riboflavin excretion values for university women students and faculty on unrestricted diets reported by Strong et al. ('41) were of the order of 500 to 800 μg. Brewer and co-workers ('46) observed the urinary excretion of riboflavin by 20 college women on self-selected diets over a three-day preliminary period. The range in urinary riboflavin was from 60 to 920 μg in 24 hours. This wide range is to be expected from women consuming different diets, although the dietary riboflavin was not determined. Similarly, Davis, Oldham and Roberts ('46) observed the urinary excretion of riboflavin when young women ingested self-chosen diets during a preliminary period. They also obtained a wide range of values, with an average daily urinary excretion of 433 μg.

Few studies showing the fecal excretion of riboflavin have been reported. Riboflavin is synthesized in the intestine, and therefore fecal excreton does not measure the unused portion of the ingested vitamin. The study of Davis and co-workers ('46) indicated that the fecal excretion of riboflavin is not

[1] Data taken from thesis presented in partial fulfillment of the requirements for the Master of Science degree in Foods and Nutrition. Present address: Alabama College, Montevallo.

markedly influenced by the dietary level unless the diet is an unusual one. Hathaway and Lobb ('46) found that a natural diet favored the synthesis of riboflavin more than did a synthetic diet, whereas Denko et al. ('46) reported that men on a diet restricted in protein and B vitamins showed a higher fecal output of riboflavin than when on a normal diet.

The purpose of this study was to determine the riboflavin value of food and excreta of young college women consuming a self-selected diet from a common food supply.

PROCEDURE

Nine young college women between the ages of 18 and 25 participated in this study. The precautions and procedures used in collecting the food and excreta were those previously reported by Holt and Scoular ('48). However, for this study the jars used for food and feces were painted on the outside with black paint to prevent light destruction of riboflavin. The amber bottles used for urine were found to give adequate protection without painting.

One Eastman Safelight lamp carrying a 20-watt bulb and equipped with a Wratten light filter was the only illumination used during the entire period of analysis.

Duplicate samples, 5 or 6 gm, of the macerated food were taken for analysis. The method employed for milk, food and feces was essentially that of Conner and Straub ('41), except that the adsorbent was washed free of dust and adsorbed gases with 2% acetic acid and repeated rinsings with distilled water, as recommended by Ferrebee ('40). The volume of the eluant necessary to free the riboflavin from the adsorbent was 80 ml for the samples used in this study, as compared to 50 used by Conner and Straub ('41). Filters B_2 and PC_2 with which the Coleman Photofluoremeter was equipped, were used for transmitting the fluorescent and incident light.

A complete blank consisting of all the reagents used throughout the method was also carried through the entire procedure. The value for the blank was subtracted from the fluorescence reading of each sample before determining the riboflavin value

from the calibration curves made with pure riboflavin. If the duplicate samples checked within a plus or minus 10%, the average was used in determining the total riboflavin content. Two curves were made, since two methods were used in determining the riboflavin values. Conner and Straub's method ('41) was used with modifications previously mentioned for the samples of milk, food and feces, while Ferrebee's method ('40) was used for the analysis of the duplicate urine samples. With the latter method it was also necessary to increase the eluant; in this case, to 60 ml from 20.

The recoveries obtained with the addition of a known amount of pure riboflavin to samples of milk, food, feces and urine prior to and during the analytical procedure ranged from 87% to 105%.

DISCUSSION

The daily riboflavin intakes and excretions of 9 young college women were determined during three 5-day periods in the late spring of 1948. Three subjects, M.W., Z.H., and J.H., were observed throughout two full periods; D.J.M., for a part of two periods; and the 5 remaining subjects participated during only one period each.

The average daily riboflavin intake and the urinary output of each subject are give in table 1. The separate analysis of milk made it possible for individual needs and preferences to be met. This also accounts for the variation in the total riboflavin intake of different subjects during the same period.

The amount of milk consumed had a definite influence on the total riboflavin intake. Subject L.D. had a consistently lower riboflavin intake than the other subjects participating in period 3 because she did not drink milk. The daily milk consumption per girl ranged from none to three glasses, contributing an average of 140 to 933 μg/day. The milk glasses used during period 3 were larger than those used previously. Also, the riboflavin content of the milk during this time was somewhat higher than in the preceding periods. This accounts for the fact that one serving of milk in period 3 contributed more riboflavin than did one serving in either of the

two other periods. The riboflavin content of the milk consumed during periods 1, 2, and 3 was 140, 120, and 200 μg/100 gm of milk, respectively. These values fall within the range reported by Theophilus and Stromberg ('45) for daily samples of milk from Jersey and Holstein cows. However the range is wider, by a small margin, than that reported by Daniel and Norris ('44).

TABLE 1

Riboflavin intake with the per cent excretion in urine

SUBJECT	AVERAGE DAILY INTAKE			AVERAGE DAILY EXCRETION	
	Food	Milk	Total	Total	Fraction
	μg	μg	μg	μg	%
	Period 1 May 10–14, 1948				
M.W.	1349 ± 401	490 ± 120	1841 ± 534	718 ± 204	38 ± 8
Z.H.	1349 ± 401	455 ± 210	1806 ± 439	670 ± 253	38 ± 14
D.J.M.	1693 ± 397	700 ± 0	2393 ± 397	1134 ± 430	46 ± 11
	Period 2 May 17–21, 1948				
M.W.	1131 ± 405	240 ± 54	1371 ± 446	478 ± 43	41 ± 9
Z.H.	1131 ± 405	240 ± 54	1371 ± 446	463 ± 72	36 ± 11
D.J.M.	1022 ± 374	675 ± 411	1447 ± 173	695 ± 53	48 ± 2
J.H.	1131 ± 405	300 ± 0	1431 ± 405	649 ± 108	51 ± 9
F.H.	1131 ± 405	300 ± 0	1431 ± 399	487 ± 21	39 ± 8
O.C.	1022 ± 374	225 ± 65	1247 ± 246	572 ± 144	51 ± 14
	Period 3 May 24–28, 1948				
J.H.	1427 ± 462	560 ± 143	1987 ± 640	817 ± 195	44 ± 15
H.B.	1427 ± 462	700 ± 0	2127 ± 462	802 ± 295	40 ± 20
L.D.	1427 ± 462	140 ± 250	1567 ± 530	587 ± 157	43 ± 18
E.B.	1152 ± 266	933 ± 165	2469 ± 689	1157 ± 197	47 ± 16

The range of the average daily riboflavin intake (food plus milk) during periods 1, 2, and 3 was 1,806 to 2,393, 1,247 to 1,447, and 1,567 to 2,469 μg, respectively. The total average daily intake during period 2 was somewhat lower than in the other two periods. This was associated with a decreased protein intake; the food protein (other than milk) averaged 65, 35 and 45 gm for the three periods. As a whole, on the days on which the riboflavin intake was less, the meats served were

fish, combination meat dishes or sandwiches. Only one subject had a negative nitrogen balance during this period. All the other subjects exhibited small nitrogen retentions. On this basis it was assumed that the previous diet, as well as the present one, was nutritionally adequate from the protein standpoint. The highest single daily intake of riboflavin from food alone occurred on the second day of period 3 when liver was the principal meat served. Tongue, custards, cheese, and foods with cream sauce also gave high riboflavin values.

The average daily urinary excretion of riboflavin ranged from 463 to 1,157 μg, with a mean average of 710 μg. This mean average agrees very well with the higher values reported by Brewer et al. ('46) and Strong et al. ('41) for young women on self-selected diets. The higher average excretion noted in the present study might be indicative of a higher intake; the intake in the above-mentioned studies was not determined. Subjects D.J.M. and E.B. of the present study consistently excreted more riboflavin in the urine than did the other subjects. These two, however, consumed a larger volume of milk than the others; consequently, the per cent of the daily intake excreted was not markedly higher.

The percentage of the average total daily intake excreted in the urine ranged from 36 to 51, with a mean average of 43%. Five subjects excreted from 46% to 51%, and 8, from 36% to 44%. These percentages are in close agreement with those reported by Everson et al. ('48) for 8 young women ingesting a daily diet supplying approximately 2.4 mg of riboflavin, although the average daily intake in the present study was lower; namely, 1.7 mg. The range in the Everson study was from 25% to 55%, with one subject excreting 55%; 6, from 33% to 43%; and one, 25%.

On the 5th day of period 2 a 5-mg supplement of riboflavin was given orally to each of 5 subjects, and the 24-hour urinary excretion was observed. The percentage of the 5-mg supplement returned (table 2) ranged from 24 to 44, with mean average of 34%. One subject returned 24% of the test dose; three, from 32% to 36%; and one, 44%. These values for

an average daily intake of 1,247 to 2,469 μg are similar to those observed by Brewer et al. ('46) at levels of 790, 1,040, 1,260, 2,230 and 2,720 μg. Their subjects were given a 3-mg test dose; they excreted 22, 30, 27, 31, and 56% in the 24 hours following the periods of controlled intake. It is interesting to note that Z.H. of the present study, who gave the lowest percentage excretion (36%) of the average total daily intake, also excreted the smallest percentage of the 5-mg supplement (24%).

The fecal output of riboflavin was determined on the three subjects of the first period and on 4 during the second period

TABLE 2

Urinary excretion of riboflavin following a 5-mg supplement

SUBJECT	AVERAGE EXCRETION PRIOR TO SUPPLEMENT	EXCRETION FOLLOWING SUPPLEMET	EXCRETION OF SUPPLEMENT
	μg	μg	%
M.W.	478	2,673	44
Z.H.	463	1,665	24
D.J.M.	695 [1]	2,288	32
J.H.	649	2,316	33
F.H.	487	2,268	36
Average	554	2,242	34

[1] Average excretion for two days only.

by administering a marker, as previously reported by Holt and Scoular ('48). The range of fecal output was greater during the first period than in the second; namely, from 515 to 954 and from 553 to 707 μg/day, respectively. These values were obtained on diets which averaged from 1,247 μg for the former to 1,447 for the latter. Only one subject (D.J.M.) participated during both of these periods. Her average output was less for the second period, 604 μg, than the 885 excreted during the first period. As previously stated, the average protein intake was also lower during the second period. The 5-mg supplement was given the last day of the second period. Even so, there was no increase in the fecal output. It is apparent from these data that the supplement

was well absorbed. These fecal riboflavin values are higher than those reported by Hathaway and Lobb ('46) for women subjects on a synthetic diet supplying 1,090 μg daily, and are lower than the excretion of the same subjects consuming a natural diet supplying 1,330 μg daily. Z.H. of the present study, who had the highest output of fecal riboflavin, had diarrhea two days during the study. While it is known that some synthesis of riboflavin takes place in the intestines, it is not known to what extent this occurs. Consequently, a true balance cannot be determined, although it is evident that the total of the average fecal output and urinary excretion of riboflavin of these 5 subjects was in all instances less than the average riboflavin intake. Therefore both dietary and supplementary riboflavin were being absorbed.

SUMMARY

The riboflavin intake in food and excretion in urine of a group of 9 college women on self-selected diets were determined chemically. The fecal output of 5 of these subjects was also determined.

The average daily riboflavin intake, exclusive of the supplement given in one experimental period, ranged from 1,247 to 2,469 μg.

The average daily urinary excretion of riboflavin on these intakes ranged from 36% to 51% of the intake.

Following a 5-mg supplement of riboflavin, one subject returned 24%; three returned 32% to 36%; and one returned 44%.

The average daily fecal output of 5 subjects ranged from 515 to 954 μg, with a mean average of 682 μg.

The total of the average daily fecal output and urinary excretion of riboflavin was less than the intake in all instances.

LITERATURE CITED

BREWER, WILLA, THELMA PORTER, RUTH INGALLS AND MARGARET OHLSON 1946 The urinary excretion of riboflavin by college women. J. Nutrition, *32:* 583.

CONNER, R. T., AND G. H. STRAUB 1941 Combined determination of riboflavin and thiamin in food products. Ind. Eng. Chem., Anal. Ed., *13:* 385.

DANIEL, LOUISE, AND L. C. NORRIS 1944 Riboflavin content of milk and milk products. Food Res., *9:* 312.

DAVIS, MARGARET V., HELEN G. OLDHAM AND LYDIA J. ROBERTS 1946 Riboflavin excretion of young women on diets containing varying levels of the B vitamins. J. Nutrition, *32:* 143.

DENKO, C. W., W. E. GRUNDY, N. C. WHEELER, C. R. HENDERSON, H. H. BERRYMAN, T. E. FRIEDEMAN AND J. B. YOUMANS 1946 The excretion of B complex vitamins by normal adults on a restricted intake. Arch. Biochem., *11:* 109.

EVERSON, GLADYS, ELIZABETH WHEELER, HELEN WALKER, AND W. J. CAULFIELD 1948 Availability of riboflavin in ice cream, peas and almonds judged by urinary secretion of the vitamin by women subjects. J. Nutrition, *35:* 209.

FERREBEE, JOSEPH W. 1940 The urinary excretion of riboflavin: Fluorimetric methods for its estimation. J. Clin. Invest., *19:* 251.

HATHAWAY, MILLICENT, AND DOROTHY E. LOBB 1946 A comparison of riboflavin synthesis and excretion in human subjects on synthetic and natural diets. J. Nutrition, *32:* 9.

HOLT, FLOWAYNE, AND F. I. SCOULAR 1948 Iron and copper metabolism of young women on self-selected diets. Ibid., *35:* 717.

STRONG, F. M., R. E. FEENEY, BARBARA MOORE AND HELEN T. PARSONS 1941 The riboflavin content of blood and urine. J. Biol. Chem., *137:* 363.

THEOPHILUS, D. R., AND O. E. STROMBERG 1945 The influence of breed, feed, and processing on the riboflavin content of milk. J. Dairy Sci., *28:* 259.

ARTIFICIAL ENRICHMENT OF WHITE RICE AS A SOLUTION TO ENDEMIC BERIBERI

I. PRELIMINARY REPORT OF FIELD TRIALS [1]

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A complete beriberi survey in Bataan Province has been reported (Salcedo et al., '48) showing an incidence of symptoms of that disease in 12.77% of 12,400 people who were clinically examined. This survey was undertaken in contemplation of the introduction of artificially enriched rice in all municipalities on the Manila Bay side of the Province (68,000 people). This was inaugurated October 1, 1948, after the installation of machinery for quantitative mixing of "premix" with rice at each of 21 rice mills in the area. Labor and depreciation on this machinery, it is estimated, will add $0.0008

[1] This study has been made possible by a grant from the Williams-Waterman Fund Committee of the Research Corporation, New York City, to the Department of Health of the Republic of the Philippines. The "premix" was donated by Hoffmann-LaRoche, Inc., Nutley, New Jersey, U.S.A. Equipment and supplies were authorized from funds allocated to the U. S. Public Health Service Rehabilitation Program in the Philippines in connection with the Philippine Rehabilitation Act of 1946 of the 79th Congress of the United States of America.

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per ganta (5 pounds) to the cost of rice. During the three months up to January 1, 1949, the enriched rice has proved acceptable to the population. The survey is to be repeated later to determine any change in incidence or in mortality from beriberi.

MATERIALS AND METHODS

Previous to the introduction of artificially enriched rice in Bataan Province, it was considered necessary to conduct preliminary field feeding trials to determine its "acceptability" in so far as color, taste, odor, palatability, and digestibility are concerned, as well as to determine any possible beneficial effects on general health and on existing peripheral neuritis and other symptoms of thiamine deficiency among the subjects tested. This was necessary in view of the disappointing experiences local public health officials had previously encountered with the introduction of brown rice, under-milled rice and certain varieties of parboiled rice. In the experiments cited below, as well as in the large-scale enrichment of rice being conducted in Bataan Province, we have used rice grains impregnated with thiamine, niacin and iron pyrophosphate, known as "premix," in such concentrations that when one part by weight of this "premix" is mixed with 200 parts by weight of white, polished rice (Furter et al., '46) the final mixture (now known as enriched rice) as delivered contains per pound 2.0 mg of thiamine, 15 mg of niacin, and 13 mg of iron. The nutrients to be used in a Philippine national enrichment program will be determined later, taking into consideration the results of present and future studies.

RESULTS AND OBSERVATIONS

Three sets of observations were made: I, among government employees; II, among children housed and fed in the Welfareville Institutions of the Social Welfare Commission; and III, among personnel of the Armed Forces of the Philippines.

I. Government employees

A single 5-lb. trial quantity of enriched rice was given to each of 284 government employees and their respective families totalling, including the employees, 2,188 persons. All of these individuals consumed the enriched rice as a part of their usual daily menus and reported uniformly that it was found satisfactory and acceptable in all instances.

II. Welfareville institutions—enriched rice observations

Two hundred eighty-seven children of both sexes and of different ages, ranging from three years to 18 years, were served enriched rice starting April 1, 1948. Another group of 287 children of similar sex and age distribution was served with the same amount of white milled rice, without "premix." The children in both groups each consumed daily 200 to 250 gm of rice in addition to the other components of their diet which, however, was generally deficient in thiamine when judged with respect to recommended daily allowances. Both groups were in fairly good health and showed no symptoms of thiamine deficiency at the start of or during the experiment. In Bataan Province it was found that clinical beriberi in children from two to 15 years of age is rare (Salcedo et al., '48). Before the project began, the children under observation were physically examined and their heights and weights were taken. Hemoglobin values and red cell counts were taken on 30 children in each group. On July 1, 1948, the same examinations were repeated to determine the effects of enriched rice on their health and nutrition.

Results of observations three months after the start of the experiment —

1. Average increase in height of the children: For the control group (no enriched rice given), 0.63 cm; for the group served with enriched rice, 0.79 cm.

2. The per cent of each group who increased in height by specified amounts is given in table 1.

3. Average gain in weight: For the control group (no enriched rice given), 0.62 kg; for the group served with enriched rice, 0.77 kg.

4. The per cent of each group who gained specified amounts in weight is given in table 2.

Comments. From the above data it may be inferred that the children served with enriched rice for a period of three months had increased in height and gained in weight more than those that were not served with enriched rice.

The above observations were continued for an additional period of 5 months, or a total of 8 months from the start, on 520 children, 260 serving as the experimental group (fed with enriched rice) and an equal number being used as controls.

TABLE 1

GROUPS UNDER OBSERVATION	NO INCREASE	INCREASE IN HEIGHT BETWEEN:			
		0.1–1 cm	1.1–2 cm	2.1–3 cm	3.1–4 cm
	%	%	%	%	%
1. Group served with enriched rice	7.66	66.50	24.00	1.84	0
2. Control group (no enriched rice)	11.84	63.76	22.64	1.76	0

TABLE 2

GROUPS UNDER OBSERVATION	NO GAIN	GAIN IN WEIGHT BETWEEN:			
		0.1–1 kg	1.1–2 kg	2.1–3 kg	3.1–4 kg
	%	%	%	%	%
1. Group served with enriched rice	9.90	63.36	21.78	4.45	0.51
2. Control group (no enriched rice)	9.42	76.63	11.43	2.52	0

Twenty-seven children from each group had to be dropped due to transfers and discharges from the institutions.

Results of observations at end of eight months —

1. Average increase in height of the children: for the control group (no enriched rice), 2.27 cm; for the group served with enriched rice, 2.34 cm.

2. The per cent of each group who increased in height by specified amounts is given in table 3.

3. Average gain in weight of the children: For the control group (no enriched rice), 1.43 kg; for the group served with enriched rice, 1.91 kg.

4. The per cent of each group who gained specified amounts in weight is given in table 4.

5. Average increase in hemoglobin %: For the control group (no enriched rice), 7.33%; for the group served with enriched rice, 13.83%.

6. Average increase in red cell count: For the control group (no enriched rice), 431,000 red cells; for the group served with enriched rice, 667,000 red cells.

Comments. From the above data it may be inferred that the children served with enriched rice for a period of 8 months had increased in height and gained in weight slightly more than those that were served with ordinary rice. It may

TABLE 3

GROUPS UNDER OBSERVATION	NO INCREASE	INCREASE IN HEIGHT BETWEEN: 0.1–1 cm	1.1–2 cm	2.1–3 cm	3.1–5 cm
	%	%	%	%	%
1. Control group (no enriched rice)	4.61	15.76	24.23	27.69	27.71
2. Group served with enriched rice	2.30	17.69	21.15	30.38	28.48

TABLE 4

GROUPS UNDER OBSERVATION	DECREASED IN WEIGHT	NO GAIN IN WEIGHT	GAIN IN WEIGHT BETWEEN: 0.1–1 kg	1.1–2 kg	2.1–3 kg	3.1–5 kg
	%	%	%	%	%	%
1. Control group	8.84	4.23	28.45	33.07	16.53	8.88
2. Group served with enriched rice	5.00	0.76	24.23	34.61	16.53	18.87

also be noted that more children among those served with enriched rice increased in height, from two to 5 cm, and gained in weight, from two to 5 kg. Fewer children among those served with enriched rice decreased in weight or gained no weight. It was also observed that enriched rice did not produce any unfavorable reactions among the children served with it.

III. Enriched rice feeding of Armed Forces personnel

A clinical survey for beriberi was conducted among the personnel of certain units of the Armed Forces of the Philippines late in September, 1947, and repeated in July, 1948, as noted in table 5.

Enriched rice was issued to units 1 and 2 (table 5) and unenriched white rice to unit 3 from January 1 to mid-July, 1948. In addition, enriched rice was issued to 800 enlisted men of the First Infantry Training Battalion and to 150 civilian employees of the Ordnance Center. Thus, a total of 1,416 persons of the military establishment consumed enriched rice and were questioned and observed as to its acceptability and its effects. Clinical observations, however, were substantially confined to the personnel listed in table 5.

The use of enriched rice was not maintained consistently at the Ordnance Center, and in all three groups there was

TABLE 5

Experimental and control groups in armed forces

UNIT	SEPT. 1947		JULY 1948		WEIGHTS [1]	
	Total men	No. with B_1 deficiency symptoms	Total men	No. with B_1 deficiency symptoms	Increased (0.5–14 lb.)	Decreased (0.5–9 lb.)
1. Signal Service Bn. (exptl.)	130	21	93	0	60	15
2. Ordnance Center (exptl.)	220	67	143	25 [2]	51	81
3. Engineer Service Bn. (control)	116	13	83	30	40	27

[1] Weight changes are recorded only for those men who remained in the units throughout the period of experiment.

[2] Sixteen cases of this group had no symptoms during the first survey.

considerable movement of men to other camps. These disturbing factors interfered with the conduct of a rigorously controlled experiment, but certain of the results appear to be of interest.

Findings. A monthly check was made on all the men receiving the enriched rice, as to any reactions to it. All stated that there was no change in taste, color or palatability. There was a mild odor reminiscent of tikitiki. There was no increase in the incidence of gastrointestinal symptoms in the daily sick call. No untoward by-effects were attributed by the men to the enriched rice. All the men of both experimental and con-

trol groups were given the other components of their usual daily diets.

DISCUSSION

The Signal Service Battalion is a unit which showed the typical reaction due to constant feeding of enriched rice for 6 months, with general improvement in the physical appearance of the men. Sixty-five per cent of the men gained in weight, while 17% lost in weight. All of those with signs or symptoms of peripheral neuritis failed to show or complain of any further manifestations of this symptom-complex. Contrast this with a unit having a big work load, much movement of personnel, and irregular feeding with enriched rice, at the Ordnance Center. In this group there were 30% who increased in weight, 55% who lost in weight. Those cases which previously showed mild symptoms and signs were relieved but, although there was a big reduction in those with one or two signs or symptoms, 16 formerly showing no symptoms had developed symptoms of peripheral neuritis after 6 months. These 16 were among personnel who had been transferred to and from other ordnance units and those on cash rations permitting them to eat outside. Considering that their messes were practically identical with those of the Signal Battalion the different results indicate the effects of neglected use of enriched rice. The results shown in the Engineer Service Battalion (controls) indicated health improvement directly attributable to improved diet (caloric increase), but the increase in the number of cases with one or two signs or symptoms of polyneuritis indicated that the quality, at least the thiamine intake, was still subnormal. Contrast this with results in the Signal Service Battalion, where all cases with symptoms improved.

Deaths from beriberi in Bataan Province

Preliminary observations on the effect on beriberi mortality in Bataan of the introduction of artificially enriched

rice are indicated in table 6. Conclusions will be drawn after a longer period of observation, and following a beriberi resurvey during 1949.

TABLE 6

Deaths from beriberi in Bataan province

(From the statistics of the District Health Officer of Bataan)

ENRICHED RICE AREA [1] (7 MUNICIPALITIES)	OCT. 1–DEC. 31 1947		JULY 1–SEPT. 30 1948		OCT. 1–DEC. 31 1948	
	Infants	Adults	Infants	Adults	Infants	Adults
1. Abucay	2	3	2	0	1	0
2. Balanga	12	1	6	1	2	1
3. Hermosa	8	2	5	1	0	0
4. Orion	7	1	6	0	5	0
5. Pilar	2	0	0	0	0	0
6. Samal	0	0	3	0	6	0
7. Orani	6	1	4	1	5	0
Totals	37	8	26	3	19	1
AREA WITH ORDINARY WHITE POLISHED RICE (5 MUNICIPALITIES)						
1. Dinalupihan	1	1	6	1	4	1
2. Bagac	1	0	2	0	1	0
3. Mariveles	0	0	0	0	0	0
4. Moron	5	0	0	1	2	2
5. Limay	0	0	5	0	2	0
Totals	7	1	13	2	9	3

[1] Enriched rice simultaneously introduced to all municipalities of this area on October 1, 1948.

SUMMARY AND CONCLUSIONS

Preliminary field trials in the Philippines with artificially enriched white rice have been made with a total of almost 3,500 individuals, including government employees and their families, members of the Armed Forces, and the children in government institutions. The results in so far as "acceptability" with reference to color, taste, odor, palatability and digestibility is concerned have been completely satisfactory. Limited observation points to beneficial effects on peripheral neuritis among members of the Armed Forces. The results

of the large-scale feeding of enriched rice in Bataan Province will be published in the future as soon as the studies are completed.

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REFERENCES

FURTER, M. F., W. M. LAUTER, E. DERITTER AND S. H. RUBIN 1946 Enrichment of rice with synthetic vitamins and iron. Ind. Eng. Chem., *38:* 486–493.

SALCEDO, JUAN, JR., E. O. CARRASCO, F. R. JOSE AND R. C. VALENZUELA 1948 Studies on beriberi in an endemic sub-tropical area. J. Nutrition, *36:* 561-577.

NUTRITION STUDIES DURING PREGNANCY

V. RELATION OF MATERNAL NUTRITION TO CONDITION OF INFANT AT BIRTH: STUDY OF SIBLINGS [1]

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FIVE FIGURES

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INTRODUCTION

Evidence that the nutrition of the human mother is important during pregnancy has become increasingly clear in recent years (Burke and Stuart, '48). Studies of the influence of maternal nutrition upon fetal growth and development, as well as investigation of its importance to the mother's health and course of pregnancy, have been and are being carried out in cooperation with the Boston Lying-in Hospital as a part of a larger research program on growth and development now in progress in the Department of Maternal and Child Health, School of Public Health, Harvard University.

Data on 216 women and their infants have already been published (Burke, Beal, Kirkwood and Stuart, '43; Burke, Harding and Stuart, '43). The present report deals with 68 younger infants born to 57 of these women. This group includes 57 second, 10 third siblings and one 4th sibling.

[1] This study was carried out under a grant from the General Education Board, Rockefeller Foundation, and is being completed under grants from the James Foundation and the Nutrition Foundation, New York City.

Data on this group of 68 siblings are compared with the published studies to learn whether in the two series a similar relationship exists between the mother's diet during pregnancy and the physical condition of her infant at birth.

Fifty-three pairs of first and second siblings [2] are presented for separate discussion because they offer a situation in which hereditary and environmental variables, in part, have been controlled. Of the 57 second siblings, all but 4 have been matched to a first sibling; 4 cases have been excluded because the mother at the first pregnancy gave birth to twins. By comparing these matched pairs of siblings it may be possible to learn whether the same mother on a like or a different diet tends to give birth to an infant in like or different physical condition. Since the kinds and amounts of food which a woman consumes are largely the result of long-established eating habits and the diets of the same mother tend to be similar in successive pregnancies, this attempt to compare the effect of maternal nutrition on matched siblings is difficult. In this paper only general ratings of the maternal diets during the latter part of pregnancy are used. No information was collected about the diets normally consumed by these women before or between pregnancies. Therefore, we do not know to what extent their food habits were changed during pregnancy except in that part of it under observation. Since no appreciable effort was made to change the food habits of these women during their pregnancies, it is likely that the dietary ratings given represent not only the diets of the women during pregnancy but, in the majority of cases, their long-time food habits. Hence, any association found between prenatal dietary rating and condition of infant at birth may be due either directly or indirectly to the diet during pregnancy, or to long-established food habits which tend to control a woman's nutritional state at the time she enters pregnancy, or to some combination of these two factors. It is possible that nutrition during the latter part of pregnancy

[2] First and second siblings refer to the order of children enrolled in the study and not necessarily to position in the family.

is a factor in certain relationships shown to exist, while long-established food habits may be important to both mother and fetus in other ways.

EXPERIMENTAL

Method of collecting and evaluating prenatal diets

The dietary histories taken from the 57 women going through one or more additional pregnancies were collected in the same manner as those in the published series (216 cases). Calculations were made based on these histories and the results evaluated in a manner already described in some detail in previous publications (Burke, Beal, Kirkwood and Stuart, '43; Burke, '47). It should be pointed out that the dietary histories give a picture of the *average* daily food intake of these women during the period studied. The ranges within the rating scale used absorb the majority of the inaccuracies which may remain in the dietary history, although our method of carefully cross checking all data obtained in the taking of the history reduces such errors to a minimum. The diets, as was the case previously, were rated in 5 categories.[3] This method of assigning a mean general dietary rating to the average daily intake of each woman during pregnancy places the women in a relatively exact relationship to each other on the basis of their average dietary intakes.

Method of rating the physical condition of the infant at birth

The pediatric ratings which describe the condition of the infants at birth and at the end of their two weeks' stay in the

[3] "Excellent," "good," "fair," "poor" and "very poor." The mean general dietary rating used in this paper was obtained in the following manner: numerical values were assigned to the ratings given each essential nutrient according to the range within which the calculated value fell: "Excellent" = 4; "good" = 3; "fair" = 2; "poor" = 1, and "very poor" = 0. The average caloric intake was also evaluated numerically. These numerical ratings were averaged and reconverted to the terminology representative of this average value of the diet. (See also footnote 5, p. 457.)

hospital nursery are based, as were those of the already published cases, upon the obstetrician's evaluation of the infant's condition at birth together with a routine physical examination by one of the pediatricians of the Growth Study Staff within 48 hours of birth and again before the infant's discharge from the hospital. The progress of the infant during his stay in the nursery was carefully recorded and any infant whose progress was not entirely satisfactory was seen frequently by one of the pediatricians on the Growth Study Staff. From this detailed information the pediatric ratings [4] for the 68 siblings were done independently by a pediatrician new to our staff.

Consideration of original 216 cases with the addition of 68 cases of siblings

Figure 1 shows the relationship between the general dietary rating for pregnancy and the physical condition of the infant at birth when the 68 cases of siblings were added to the 216 published cases and the results depicted graphically, as in the original publication (Burke, Beal, Kirkwood and Stuart, '43, fig. 2, p. 580). In the 284 cases when the maternal diet was "excellent or good," 95% of the infants were in good or excellent physical condition at birth and only 5% were in either fair or poorest physical condition. In contrast, when the maternal diet was "poor to very poor" 65% of the infants

[4] An infant was called "excellent" ("superior" in the previously published series) when he had no physical count of any kind against him either at the birth examination or during his first two weeks of life; he was called "good" with only one or possibly two minor physical counts against him. An infant was called "poorest" when he was stillborn or died during the neonatal period, had a congenital malformation at birth, was premature (under 5 lb. at birth), or was "functionally immature" (physical development and performance of the infant considered below normal in some way other than weight and length alone). All other infants were termed in "fair" condition. This group includes some infants in a fair to good condition, others in a fair to poor condition, according to the pediatric findings. In interpreting these ratings, as in the case of the infants on whom findings have been published, those infants termed "good" are relatively close to those termed "excellent" in physical condition, while the group called "fair" represents a wider range.

were in the poorest physical condition at birth and 27% were in fair condition, while only 8% were in either good or excellent physical condition. Average birth weights and lengths of infants in each dietary category are included in figure 1 and in subsequent figures.

Figure 2 represents the 216 cases with the original "fair" diet group (149 cases) divided into 103 cases which comprise

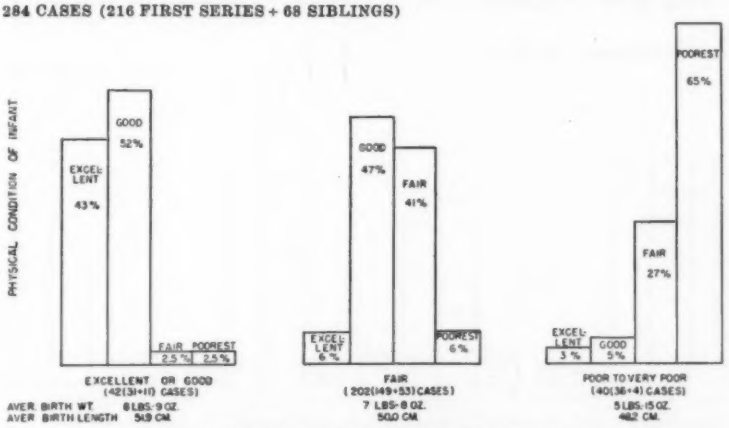

Fig. 1 Relationship of prenatal dietary rating (mean general) to the physical condition of the infant at birth. (Three maternal dietary groups, 284 cases.)

a somewhat narrower "fair" diet group; the remaining 46 cases are called "poor."[5] This division was made since further work led us to believe that the relationship between the condition of the fetus and a poor nutritional environment in the mother changes most abruptly as the diet becomes very poor. The lowest mean general dietary ratings are then

[5] The original "fair" diet group contained all mean general dietary ratings from 2.7 to 1.1 inclusive. In dividing this rather wide "fair" diet group, the new "fair" diet group was made to include only mean general dietary ratings from 2.7 to 1.8 inclusive. The ratings from 1.7 to 1.1 inclusive are termed "poor." The lowest mean general dietary ratings, as was the case previously with those under 1.1, are called "very poor." Mean general dietary ratings from 2.8 to 3.2 inclusive are termed "good" and of 3.2 or more "excellent."

termed "very poor" and represent an extremely deficient dietary even in the minds of the most conservative. The division into 4 dietary groups, used in figure 2, shows more clearly the consistent trend toward lower physical ratings of infants with lower maternal dietary ratings, and particularly the abrupt increase in the percentage of poorest infants as the diet changes from "poor" to "very poor." It is evident that a "very poor" maternal diet is associated with an infant in the

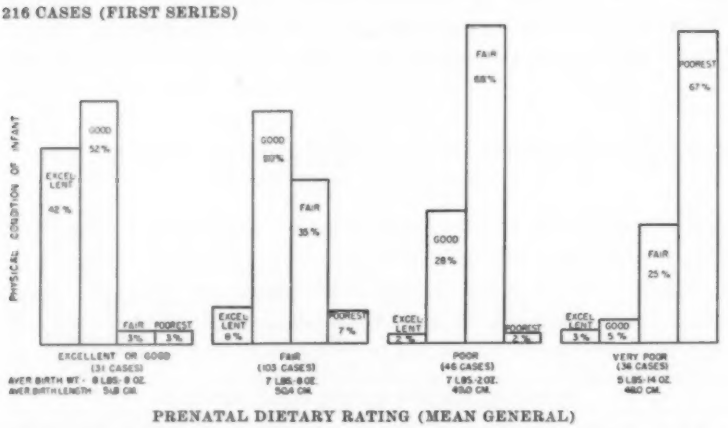

Fig. 2 Relationship of prenatal dietary rating (mean general) to the physical condition of the infant at birth. (Four maternal dietary groups, 216 cases, first series.)

poorest physical condition at birth, while an "excellent or good" diet is associated with an infant in excellent or good condition at birth. Furthermore, figure 2 illustrates the consistent shift in physical condition of the infant with each change in either direction in the mean general prenatal dietary rating.

Figure 3 presents the data on the 68 siblings in like manner. An essentially similar graph results, except that the "poor" diet group contains a considerably higher incidence of poorest infants than was found in the original 216 cases.

Relation of average birth weights and birth lengths of infants to prenatal dietary ratings (mean general)

The average birth weights and lengths of the infants in the various maternal dietary categories are given in each figure shown. In every instance the average birth lengths and weights decrease as the maternal dietary ratings become poorer. The constancy of this relationship in every grouping of cases is impressive.

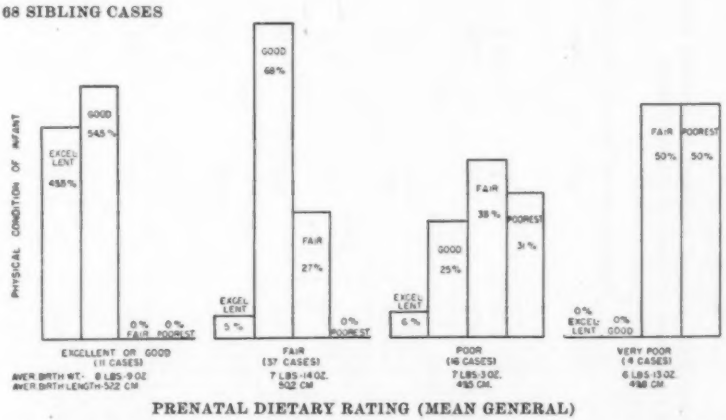

Fig. 3 Relationship of prenatal dietary rating (mean general) to the physical condition of the infant at birth. (Four maternal dietary groups, 68 sibling cases.)

Since physical condition of infant and birth weight and length are to some extent related, and physical condition of infant at birth and maternal dietary ratings are closely related, the question arises: Are birth weight and birth length more closely associated with physical condition of infant at birth than with maternal dietary rating? Table 1 attempts to answer this question by listing average birth weights and lengths of infants according to the prenatal dietary ratings and according to the physical condition of the infants at birth. Although a study of this table demonstrates a relationship

TABLE 1

Average birth weights and birth lengths of infants (284) grouped according to prenatal dietary rating (mean general) and according to physical condition of infant at birth

PHYSICAL CONDITION OF INFANT AT BIRTH	AVERAGE BIRTH WEIGHT AVERAGE BIRTH LENGTH	PRENATAL DIETARY RATING — MEAN GENERAL					
		Excellent	Good	Fair	Poor	Very poor	Total no. cases
Excellent	Weight lb. oz.	(10)[1] 8–10	(8) 8–9	(10) 7–10	(2) 8–2	(1) 6–10	31
	Length cm	52.2	51.2	50.5	52.2	50.3	
Good	Weight lb. oz.	(3) 9–7	(19) 8–6	(77) 7–13	(17) 7–3	(2) 6–8	118
	Length cm	52.6	51.9	50.5	48.9	47.6	
Fair	Weight lb. oz.	(0) ...	(1) 7–4	(46) 7–8	(37) 7–1	(11) 6–8	95
	Length cm	...	51.2	50.3	(36) 48.9[2]	48.8	
Poorest	Weight lb. oz.	(1) 8–11	(0) ...	(7) 6–10	(6) 7–3	(25) 5–10[3]	40
	Length cm	52.6	...	48.6	51.3	(22) 47.8[2]	
Total no. cases		14	28	140	62	40	284 (216 + 68)

[1] Numbers in upper left hand corners = number of cases.
[2] Four cases no birth lengths.
[3] One case no birth weight.

between physical condition at birth and birth weight and length, a closer relationship is shown between maternal dietary rating for pregnancy and birth weight and length of infant.

Comparison of 53 matched pairs of siblings

Figure 4 depicts the relationship between the maternal dietary ratings and the physical condition of the first siblings

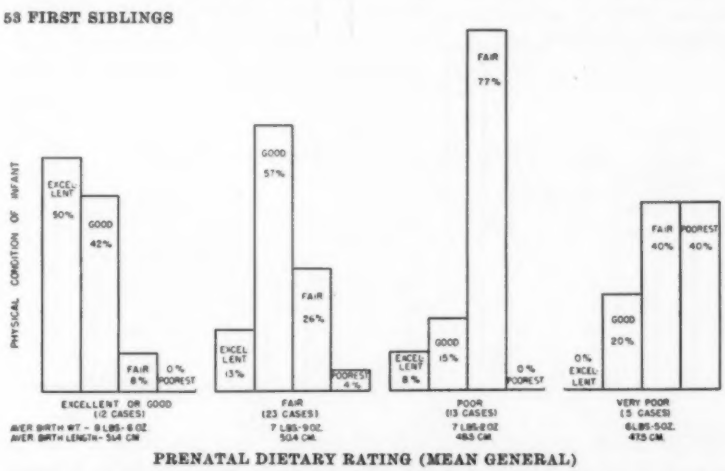

Fig. 4 Relationship of prenatal dietary rating (mean general) to the physical condition of the infant at birth. (Four maternal dietary groups, 53 first siblings.)

of the 53 matched cases (taken from the 216 original cases). Figure 5 shows the same relationship for the second siblings, younger brothers or sisters of the first siblings. These figures illustrate again in a striking manner the consistent shift in the physical condition of the infant with each change in either direction in the mean general prenatal dietary rating.

The relationship between the prenatal dietary ratings of the 53 mothers during their first and second pregnancies was studied. Thirty of the 53 women had the same dietary ratings during both pregnancies. Six of the mothers improved their

dietary ratings by one category and 5 improved them by two categories. On the other hand, 7 of the mothers had dietary ratings during their second pregnancies which were poorer by one category than those during the first, while 5 showed ratings which were worse by two categories. The group as a whole remained at about the same dietary level during their two pregnancies. It is well known that to change an individual's food habits is time-consuming and costly. Apparently greater motivation and more education are required

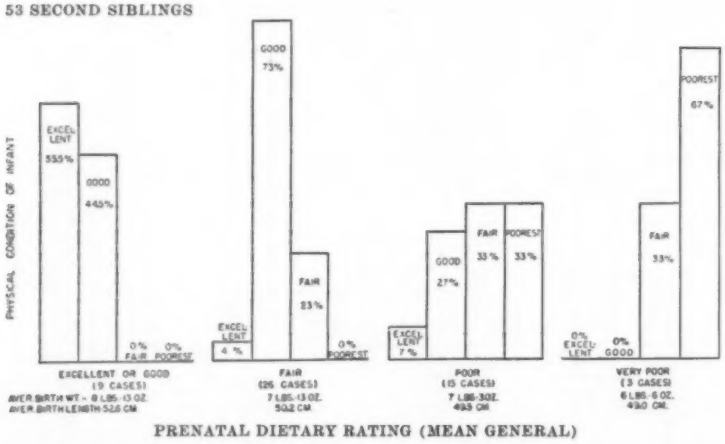

Fig. 5 Relationship of prenatal dietary rating (mean general) to the physical condition of the infant at birth. (Four maternal dietary groups, 53 second siblings matched to 53 first siblings.)

than were given to this group of women. It is unfortunate for this study that the diets of these women tended to remain the same or changed only slightly during their successive pregnancies.

The physical ratings of the first and second siblings of the 53 mothers are shown in table 2. These mothers tended to produce pairs of offspring in the same condition during their successive pregnancies. It should be noted that these 53 mothers, who enrolled two or more of their children in the

Growth Study, produced better babies than did the remainder of the 216 mothers. There were neither stillbirths nor neonatal deaths in the 106 children involved here.

Table 3 lists all the poorest first and second siblings in the 53 matched pairs, and includes an explanation of their physical ratings. It compares each infant with his sibling and with the dietary ratings of their mother during her two pregnancies. It is of interest that of the 12 poorest infants 4 comprised two pairs of siblings. Of the remaining 8 poorest siblings, 5 are paired with a brother or sister in fair condition. In only three instances was a poorest sibling paired with an infant in good or excellent physical condition.

TABLE 2

Physical ratings at birth on first and second siblings

RATINGS ON SECOND SIBLINGS	RATINGS ON FIRST SIBLINGS				
	Exc.	Good	Fair	Poorest	Total
Excellent	*1*	5	1	0	7
Good	7	*12*	6	2	27
Fair	1	3	7	1	12
Poorest	1	1	3	*2*	7
Total	10	21	17	5	53

Table 4 shows the change in the condition at birth of the second sibling from that of his older brother or sister with the change in the mean general prenatal dietary rating. The diet was considered to have changed for better or worse if it was shifted one or more dietary categories (i.e., from "good" to "excellent," etc.). Eleven mothers improved their diets during their second pregnancies. Six of these produced second infants which were in better condition than the first, while only three produced second babies in worse condition. Among 12 mothers whose diets were worse during their second pregnancies, 5 produced second babies in worse condition, while only two produced better infants.

It has been pointed out previously that there were 10 mothers whose diets had changed by two categories. It is of

some interest to see what happened to the respective babies. There were three mothers whose diets shifted from fair to excellent: in these cases the condition of the babies changed from good to excellent. There were two mothers whose diets changed from very poor to fair: in one of these cases the condition of the second sibling improved from poor to fair, while in the other the condition of both babies was good. There were 5 mothers whose diets during their second preg-

TABLE 3

Comparison of all poorest first and second siblings in the 53 matched pairs with their sibling and with the dietary ratings of the mother during the two pregnancies

CASE NO.		PHYSICAL CONDITION OF INFANT AT BIRTH	PRENATAL DIETARY RATING (mean general)
First sibling	Second sibling		
50		Fair	Very poor
	130	Poorest — functionally immature	Poor
32		Fair	Poor
	131	Poorest — hemorrhagic disease of newborn	Poor
56		Fair	Poor
	145	Poorest — hemorrhagic disease of newborn	Poor
24		Excellent	Poor
	170	Poorest — congenital malformation of ribs	Poor
250		Good	Fair
	270	Poorest — club foot, hammer toe	Poor
116		Poorest — bifid uvula	Fair
	214	Poorest — cleft palate (feeble-minded at 1 yr.)	Very poor
158		Poorest — premature (3 lb. 14 oz.)	Very poor
	228	Poorest — premature (3 lb. 15 oz.)	Very poor
81		Poorest — congenital heart (heart murmur prominent during first year; rapidly disappeared thereafter)	Excellent
	239	Fair	Good
231		Poorest — cleft palate (feeble-minded at 1 yr.)	Very poor
	285	Fair	Fair
191		Poorest — premature (3 lb. 6 oz.), inguinal hernia	Fair
	305	Good	Fair

nancies were worse by two categories than those during the first pregnancies. For 4 of these the dietary change was from excellent to fair. The condition of the infant in two of these cases deteriorated, it remained the same in one case, and the second infant was better than the first in the other case. There was one mother whose diet shifted from fair to very poor and both of her infants were rated poorest at birth.

Improvement of the infant's condition with improvement of the maternal diet and deterioration of the infant's condition with deterioration of the maternal diet appear in this series where each of the 53 mothers was observed through two pregnancies, but the changes are not sufficiently striking

TABLE 4

Change in condition of infant with change in maternal dietary rating (mean general)

SECOND MATERNAL DIETARY RATINGS	SECOND SIBLINGS			
	Better	Same	Worse	Total
Better	*6*	2	3	11
Same	7	*15*	8	30
Worse	2	5	*5*	12
Total	15	22	16	53

to be statistically significant. Nevertheless, this particular series of matched siblings is unique since it has been possible to use each mother as her own control and thus to eliminate to some extent certain of the extraneous factors which influence the condition of babies at birth.

SUMMARY AND CONCLUSIONS

A study of the physical condition at birth of 68 siblings (57 second, 10 third siblings and one fourth sibling) born to 57 of the women studied in a previously published series of cases has been made in relation to the mean general dietary rating for the latter part of these subsequent pregnancies.

1. In the 284 cases when the maternal diet was "excellent or good," 95% of the infants were in good or excellent physi-

cal condition at birth and only 5% were in either fair or poorest physical condition. In contrast, when the maternal diet was "poor to very poor" 65% of the infants were in the poorest physical condition at birth and 27% were in fair condition, while only 8% were in either good or excellent physical condition.

2. The "fair" diet group was divided into two groups, thus giving 4 maternal dietary groupings: (1) "excellent or good"; (2) "fair"; (3) "poor"; and (4) "very poor." This has helped to make clear that a "very poor" maternal diet is associated with an infant in the poorest physical condition at birth, while an "excellent or good" diet is associated with an infant in excellent or good condition at birth. The consistent shift in physical condition of the infant with each change in dietary rating in either direction is impressive.

3. The statement still remains true that all of the stillborn infants, all of the neonatal deaths but one, all of the premature infants but one (a neonatal death), all of the functionally immature infants, and most of the congenital defects were found in the group of infants born to mothers with "poor" or "very poor" prenatal diets. Since no major effort was made to change the dietary habits of these women, it is probable that in the majority of the cases the prenatal dietary rating was representative also of long-time food habits.

4. The average birth weight and birth length of the infant decreased as the maternal dietary rating became poorer. The constancy of this relationship is impressive. While a relationship exists between physical condition of infant and birth weight and length, a closer relationship is shown between maternal dietary rating for pregnancy and birth weight and length of infant.

5. Data were presented on 53 matched pairs of siblings. Improvement of the infant's condition with improvement of the maternal diet and deterioration of the infant's condition with deterioration of the maternal diet appeared in this series where each of the 53 mothers was observed through two pregnancies. While the changes were not sufficiently striking to

be statistically significant, it is felt that this series of matched siblings is unique since it has been possible to use each mother as her own control and thus in part minimize hereditary and environmental variables which influence the condition of babies at birth. Similar studies of women through successive pregnancies may help further to clarify the relationship between prenatal diet and the condition of the infant at birth.

LITERATURE CITED

BURKE, B. S. 1947 The dietary history as a tool in research. J. Am. Diet. Assoc., *23:* 1041.

BURKE, B. S., V. A. BEAL, S. B. KIRKWOOD AND H. C. STUART 1943 The influence of nutrition during pregnancy upon the condition of the infant at birth. J. Nutrition, *26:* 569.

BURKE, B. S., V. V. HARDING AND H. C. STUART 1943 Nutrition studies during pregnancy: IV. Relation of protein content of mother's diet during pregnancy to birth length, birth weight and condition of infant at birth. J. Pediat., *23:* 506.

BURKE, B. S., AND H. C. STUART 1948 Nutritional requirements during pregnancy and lactation. J. Am. Med. Assoc., *137:* 119.

FOLIC ACID STUDIES IN THE MINK AND FOX[1]

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TWO FIGURES

(Received for publication March 11, 1949)

Previous publications from this laboratory have shown that mink and foxes require a dietary source of folic acid (Schaefer et al., '46, '47). Later it was shown that an unidentified factor present in a 60% methanol extract of fresh liver is also needed (Schaefer, Whitehair and Elvehjem, '48). Since this extract was found to contain only 2.0 to 2.3 μg of folic acid per milliliter,[3] and in view of the similarity of syndrome of the two deficiency states, we undertook a study of the interrelationship between these two dietary essentials. An earlier observation on the inability of the fox to use the conjugated forms of folic acid was studied further through the use of crystalline materials.

EXPERIMENTAL AND RESULTS

Four fox pups and 29 mink kits were placed on a purified basal ration which had the following composition in grams: sucrose 66, casein[4] 19, salts IV (Phillips and Hart, '35) 4,

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A portion of this work was reported briefly in Fed. Proc., *8:* 399, 1949.

[2] Present address: Agricultural Experiment Station, Alabama Polytechnic Institute, Auburn, Alabama.

[3] We are indebted to O. E. Olson for the microbiological assay of folic acid in this material.

[4] Smaco vitamin test.

and cottonseed oil 11. Each 100 gm ration was supplemented with 0.2 mg thiamine HCl, 0.2 mg pyridoxine HCl, 0.4 mg riboflavin, 1.5 mg Ca pantothenate, 4.0 mg nicotinic acid, 100 mg choline, 25 mg *i*-inositol, 50 mg *p*-aminobenzoic acid, 0.5 mg 2-methyl 1-4 naphthoquinone, and 0.025 mg biotin. Haliver oil fortified with α-tocopherol acetate and vitamin D_3 was added so that each 100 gm ration contained 4 mg α-tocopherol, 1,200 I. U. vitamin A and 120 I. U. vitamin D_3. The care and handling of the animals were the same as in previous experiments.

Fox studies

After receiving the basal ration for 13 weeks, a loss of body weight (fig. 1) and a decrease in hemoglobin content of the blood were noted in fox 52. Thirty milliliters of a commercial refined liver extract [5] were given parenterally in doses of 3 ml every third day. No response in body weight or hemoglobin resulted, and the ration was supplemented with 0.1 mg folic acid per 100 gm ration. Body weight increased 1.26 kg in 10 weeks and the hemoglobin increased 2.5 gm% in the same period of time.

When fox 58 (fig. 1) had been on the basal ration 12 weeks a decline in body weight occurred, and three weeks later anemia and anorexia developed. Five milliliters of the methanol extract of fresh liver (1 ml = 10 gm liver) was administered orally and the anorexia was corrected. The methanol extract was then included in the ration at a level of 1% (1 ml per 100 gm ration), and an immediate response in body weight followed. The hemoglobin, which had varied between 12.8 and 12.1 gm%, increased to 15.5 when the methanol extract was given.

After foxes 62 and 63 (fig. 1) had received the basal ration for 18 and 14 weeks respectively, the syndrome of folic acid deficiency developed. Six milligrams of folic acid as the heptaglutamate were injected intravenously (2 mg every

[5] Sharp and Dohme No. 2505, 15 U.S.P. antipernicious anemia units per milliliter.

other day) into the latter animal, but loss of body weight and hemoglobin continued. Two milligrams of folic acid were given by intravenous injection and 18 days later an additional 2 mg were administered. A prompt increase in body weight and hemoglobin content of the blood resulted. In fox 62, 2.5 mg folic acid as the heptaglutamate were injected intravenously, and when it was evident that no response

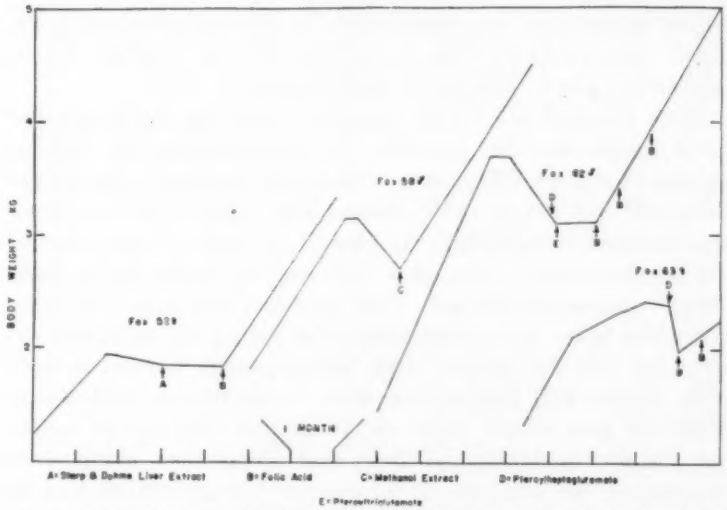

Fig. 1 Growth curves for foxes receiving the basal diet and the basal diet supplemented with folic acid and liver extracts.

would be obtained 5 mg of pteroyl-triglutamic acid [6] were also administered. Hemoglobin levels remained between 10.7 and 11.5 gm%, and no increase in body weight was noted. Twenty-six days later 2 mg folic acid were given parenterally, and two more injections at the same level were given at two-week intervals. Three months after receiving the first injection of folic acid, body weight had increased 2.9 kg, but hemoglobin remained at relatively the same level.

[6] "Teropterin."

Mink studies

Mink 74 (table 1), receiving the folic acid-"free" ration, grew normally for 7 weeks and then failed to grow. When 1% methanol extract was added to the diet, body weight increased 325 gm in 8 weeks and the hemoglobin increased 3.6 gm%. A second decline in body weight and hemoglobin then occurred even though the extract was continued. Five milligrams of folic acid administered parenterally produced no improvement and the animal died. In light of previous work, death was probably due to a lack of the residue factor (Schaefer, Tove, Whitehair and Elvehjem, '48).

Mink 175 and 177 (table 1), after receiving the basal diet for 8 weeks, lost 500 and 430 gm, respectively. The former animal was given 1% of the methanol extract and body weight increased 310 gm in three weeks. The same level of extract was injected intramuscularly (based on food consumption of the previous day) into mink 177, and in three weeks body weight increased 300 gm. Two animals, 109 and 107, were given the basal ration supplemented with 1 ml methanol extract per 100 gm ration. Mink 109 appeared normal in both body weight and hemoglobin after 7 months on experiment. Mink 107 lost weight after receiving this diet for 16 weeks, but the administration of folic acid either in a single dose of 1 mg or the addition of 0.1 mg per 100 gm ration had no effect. A response was obtained, however, upon the addition of 4% residue to the ration.

Mink 134 and 157 exhibited deficiency symptoms after receiving the basal ration for 4 and 8 weeks, respectively. Parenteral administration of a commercial liver concentrate [7] equivalent to five-tenths or one antipernicious anemia unit in the former animal and five-tenths unit in the latter, had no effect. However, when the methanol extract was given, a response in body weight resulted. The daily injection of 1 μg vitamin B_{12} for 5 days was ineffective in alleviating symptoms of a lack of the methanol extract factor exhibited by

[7] Eli Lilly and Company. Reticulogen, 20 U.S.P. antipernicious anemia units per milliliter.

mink 178. Vitamin B_{12} also failed to elicit a response in mink 142 receiving 4% residue in addition to the basal ration supplemented with folic acid.

TABLE 1

Effect of various supplements on growth of mink receiving the basal ration

MINK NO.	RATION	DAYS ON EXPERIMENT	BODY WEIGHT	SUPPLEMENT	DAYS	BODY WEIGHT
			gm.			gm.
74	Basal	50	595	1% MeOH ext.	59	920
175	Basal	56	770	1% MeOH ext.	21	1080
177	Basal	59	865	1% MeOH ext.	21	1165
109	Basal + 1% MeOH ext.	182	1170	.....	..	....
107	Basal + 1% MeOH ext.	114	850	1 mg folic acid [1]	12	835
				0.1 mg folic acid/100 gm	42	775
134	Basal	29	695	0.025 ml Reticulogen/day [2]	27	760
				0.05 ml Reticulogen/day	21	740
				1% MeOH ext.	14	695
				2% MeOH ext.	63	1135
157	Basal	63	810	0.025 ml Reticulogen/day	21	785
				1% MeOH ext.	77	1250
178	Basal + 0.1 mg folic acid	86	1030	1 μg B_{12}/day [3]	5	910
				1% MeOH ext.	6	970
				1% MeOH ext.	42	1215
142	Basal + 0.1 mg folic acid + 4% residue	69	825	1 μg B_{12}/day [3]	4	740
				1% MeOH ext.	25	1020

[1] Single dose injected intramuscularly.

[2] Refined liver extract containing 20 U.S.P. antipernicious anemia units per milliliter, injected intramuscularly.

[3] Given daily by intramuscular injection.

The effect of a crude antagonist of folic acid (7-methyl folic acid) was studied with mink 76, 82 and 112 (fig. 2). Three-tenths per cent of the antagonist was added to the basal diet supplemented with 0.1 mg folic acid per 100 gm ration. One per cent methanol extract was also added to the ration of mink 76. Immediate loss of body weight occurred in animals 82 and 112, and when 1% methanol extract was added to the ration an increase in body weight resulted. The response was not complete and a second decline occurred.

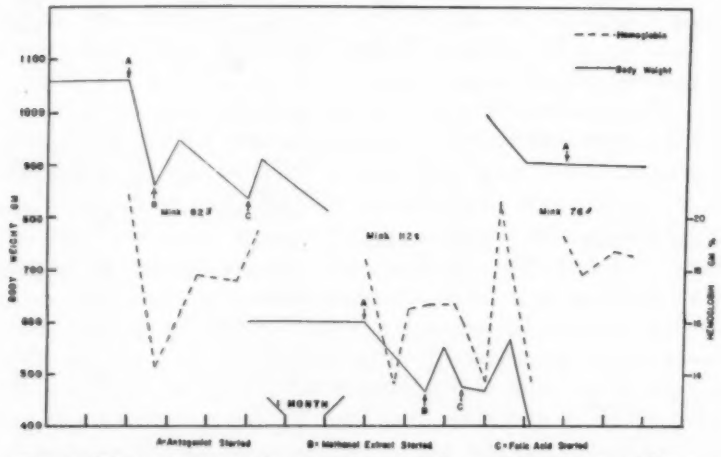

Fig. 2 Effect of folic acid antagonist on body weight and hemoglobin content of blood of mink.

The folic acid was increased to 0.2 mg per 100 gm ration and a second response was obtained, although the body weight did not return to a normal value. No change in body weight was observed in mink 76, which received the extract from the start.

There was a marked decrease in hemoglobin in animals 82 and 112 upon the addition of the antagonist. In the former animal the addition of the extract resulted in an increase of 3.7 gm%. When body weight declined the second time the hemoglobin also fell slightly, but rose again when the folic acid

content of the ration was increased. In mink 112, however, hemoglobin rose spontaneously before the extract was given, and the addition of the methanol extract produced no further increase. After the folic acid content of the ration was raised, the hemoglobin level declined but rose again as body weight increased. In the third animal, mink 76, the hemoglobin level remained quite normal.

The folic acid content of the blood, urine and feces of mink receiving the basal ration supplemented with either folic acid or the methanol extract or both, was determined. The assay used was the turbidimetric method of Luckey, Briggs and Elvehjem ('44) with *Streptococcus faecalis* (American Type Culture Collection No. 8043). Since it had been observed [8] that the incubation of blood with buffer at pH 7 resulted in an increase of free folic acid as great as that obtained when enzyme preparations were added, the former procedure was adopted. Accordingly, blood samples were prepared in the following manner.[9]

Three or 4 ml oxalated blood obtained by cardiac puncture were added to 5 ml pH 7.0 buffer and incubated under toluene for 4 hours at 37°C. A crystal of meta phosphoric acid was added and the sample placed in a boiling water bath for three minutes. It was then cooled, neutralized, diluted to a suitable volume and filtered.

Twenty-four-hour samples of urine and feces were collected by placing the animals in metabolism cages. Food consumption records were kept. The urine was collected in dark bottles containing toluene, diluted to a suitable volume and filtered for assay. Feces were homogenized and allowed to autolyze under toluene 72 hours at 37°C., and then autoclaved for 15 minutes at 15 lb. pressure. Folic acid was released enzymatically by incubating the sample first with a hog pancreas preparation and then with a hog kidney enzyme prepa-

[8] Olson, O. E., E. E. C. Fager, R. H. Burris and C. A. Elvehjem, unpublished data.

[9] This procedure was developed by O. E. Olson and modified by A. Sreenivasan and A. E. Harper at this laboratory.

ration. The general procedure of Sreenivasan, Harper and Elvehjem ('49) was followed.

The results of these assays are presented in table 2. The addition of 1% methanol extract to the basal ration produced the same increase in the folic acid content of blood, urine and feces as did supplementing the diet with 0.1 mg folic acid per 100 gm ration. While the methanol extract did not increase the blood folic acid of animals receiving 0.1 mg folic acid per 100 gm ration, it did produce an increase in the urine and fecal levels.

TABLE 2

Effect of methanol extract of fresh liver on folic acid content of blood, urine and feces of mink

RATION	SERIES 1				SERIES 2			
	No. mink	Blood folic acid	No. mink	Urine folic acid mμg/gm ration consumed	No. mink	Urine folic acid mμg/gm ration consumed	No. mink	Fecal folic acid μg/gm ration consumed
		mμg/ml						
Basal	4	< 1.95	2	1.45	.	....	2	< 0.19
Basal + 0.025 mg folic acid per 100 gm ration	3	< 1.80	4	2.60	5	3.03	1	0.27
Basal + 0.1 mg folic acid per 100 gm ration	7	3.80	5	2.81	7	3.70	2	0.82
Basal + 1% MeOH ext.	3	3.65	1	2.06	4	5.98	1	0.99
Basal + 0.1 mg folic acid per 100 gm ration + 1% MeOH ext.	3	3.32	1	4.60	2	4.09	1	3.88

DISCUSSION

Since the methanol extract, which contains only 2 μg folic acid per milliliter, will replace folic acid in the ration of the mink and the fox and reverses the crude folic acid antagonist in the mink, it becomes apparent that the methanol extract contains a factor which has a sparing action on folic acid. Further support is given this hypothesis by the fact that the blood, urine and fecal levels of folic acid increased when the methanol extract was added to the ration.

Our results confirm the observations of Cartwright et al. ('48) and Cunha et al. ('48), with pigs, that antipernicious anemia liver extracts fail to replace folic acid. The inactivity of these commercial liver extracts and of crystalline B_{12} indicates that the methanol extract factor is different from vitaman B_{12}.

Day and Totter ('48) have reported that only small amounts of the folic acid fed to monkeys are recovered in the urine. A similar observation with respect to pterioc acid in humans was made by Franklin, Stokstad and Jukes ('47). The results of the folic acid assays in our experiments reveal the same situation applying to the mink.

The earlier observation that folic acid conjugate (as yeast) failed to replace folic acid for the fox has been confirmed by the use of the crystalline compound. Since the conjugate was injected intravenously, it would appear that the animal is incapable of hydrolyzing the heptaglutamate compound and that the inactivity is not due merely to lack of absorption of a large molecule or lack of hydrolysis within the intestinal tract. Preliminary results indicate that pteroyl-triglutamic acid is also inactive.

SUMMARY

1. A methanol extract of liver contains a factor which has a sparing action on the dietary folic acid requirement of the mink.

2. The effect obtained with the methanol extract is not obtained with vitamin B_{12} under our experimental conditions.

3. Folic acid conjugates are incapable of replacing folic acid for the fox.

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LITERATURE CITED

CARTWRIGHT, GEORGE E., JANE FAY, BETTY TATTING AND MAXWELL M. WINTROBE 1948 Pteroyl glutamic acid deficiency in swine; effects of treatment with pteroyl glutamic acid, liver extract and protein. J. Lab. Clin. Med., *33:* 397.

CUNHA, T. J., R. W. COLBY, L. K. BUSTAD AND J. F. BONE 1948 The need for and interrelationship of folic acid, antipernicious anemia liver extract and biotin in the pig. J. Nutrition, *36:* 215.

DAY, PAUL L., AND JOHN R. TOTTER 1948 Pteroyl-glutamic acid balance studies in monkeys. Ibid., *36:* 803.

FRANKLIN, A. L., E. L. R. STOKSTAD AND T. H. JUKES 1947 Urinary excretion studies following the administration of pteroic acid. Proc. Soc. Exp. Biol. Med., *66:* 576.

LUCKEY, T. D., G. M. BRIGGS, JR. AND C. A. ELVEHJEM 1944 The use of *Streptococcus lactis R* for the measurement of folic acid. J. Biol. Chem., *152:* 157.

PHILLIPS, P. H., AND E. B. HART 1935 The effect of organic dietary constituents upon chronic fluorine toxicosis in the rat. Ibid., *109:* 657.

SCHAEFER, A. E., S. B. TOVE, C. K. WHITEHAIR AND C. A. ELVEHJEM 1948 The requirement of unidentified factors for mink. J. Nutrition, *35:* 157.

SCHAEFER, A, E., C. K. WHITEHAIR AND C. A. ELVEHJEM 1946 Purified rations and the importance of folic acid in mink nutrition. Proc. Soc. Exp. Biol. Med., *62:* 169.

———— 1947 Purified rations and the requirement of folic acid for foxes. Arch. Biochem., *12:* 349.

———— 1948 Unidentified factors essential for growth and hemoglobin production in foxes. J. Nutrition, *35:* 147.

SREENIVASAN, A., A. E. HARPER AND C. A. ELVEHJEM 1949 The use of conjugase preparations in the microbiological assay of folic acid. J. Biol. Chem., *177:* 117.

THE IRON METABOLISM OF YOUNG WOMEN ON TWO LEVELS OF INTAKE

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Additional information is needed regarding the iron requirement of women. The National Research Council ('48) recommendation for an intake of 12 mg per day was arrived at by making an estimate of the requirement and adding an amount supplying a factor of safety. There is little likelihood that further work will demonstrate that this allowance should be raised, but more work needs to be done to test lower levels of intake. A study of the iron metabolism of young women carried out in this laboratory included, along with an investigation of the absorption of iron from beef (Johnston, Frenchman and Boroughs, '48) which has already been reported, a study of the adequacy of two levels of iron intake. One level was much lower than the recommendation of the National Research Council, and one approached it.

EXPERIMENTAL

Five young women 18 years of age were maintained for 10 weeks on a controlled diet. The total period was divided into a two-week adjustment period and two experimental periods each 4 weeks long. During the adjustment period and the first experimental period the diet contained 7 mg of iron a day, and during the second experimental period it contained 10.4 mg per day. The basal diet for all 10 weeks was

of a dietary pattern considered adequate by nutritionists; it included in each day one serving of a flesh food, one egg, one quart of milk, two servings of vegetables besides potatoes, and two or more servings of fruit. All the bread was made from an especially prepared unenriched flour.[1] To avoid a high phytic acid content the diet contained no whole cereals, shelled beans, nuts or cocoa and only one chocolate flavored food a week. The only change made in the diet when the iron content was increased to 10.4 mg was the addition of two 100-gm patties of ground beef made up of one-third fat and two-thirds lean meat; one was given in the breakfast and the other in the lunch. This resulted in a diet containing meat in every meal.

Composites of food were made each week for the entire 10 weeks; composites of stools were made for each week except for the two preliminary weeks; composites of urine were made for only a 4-day period because the excretion of iron in the urine is constant for a given individual and is so small as to be of little importance; composites of the menses were made for each period occurring during the 10 weeks. The method of collection, the preparation for analysis and the analyses of the foods and stools were described in the previous paper (Johnston, Frenchman and Boroughs, '48). The urines were treated with concentrated HCl and bottled for analysis at a later date. The menses were collected on tampon-type pads and the pads were dropped into redistilled water and let stand for several hours, after which enough HCl was added to make a final concentration of 10% and the whole heated until the pads disintegrated. A correction was made for the iron content of the pads. The urine and menses were analyzed in the same way as the foods and stools: they were ashed with sulphuric and nitric acids, and iron determined by the thiocyanate method of Stugart ('31).

[1] The flour was especially prepared for this project without enrichment by the Pillsbury Mills, Inc.

RESULTS

Retentions were calculated by subtracting the iron content of the urine and stools from the iron of the foods. Mean iron retentions per day for 4 weeks on an intake of approximately 7 mg of iron were 0.26, 0.43, 0.61, 0.64 and 1.33 mg (table 1) for the 5 subjects. After beef was added to raise the intake to approximately 10.4 mg, the retentions increased to 1.77, 1.79, 1.80, 2.37 and 2.53 per day (table 2).

TABLE 1

Retentions of iron on an intake of approximately 7 mg a day for 4 weeks

SUBJECT	INTAKE	EXCRETION		RETENTION
		Urine	Feces	
	mg/day	*mg/day*	*mg/day*	*mg/day*
A	6.90	0.06	6.20	0.64
B	7.00	0.06	6.68	0.26
C	7.08	0.07	6.58	0.43
D	6.53	0.08	5.84	0.61
E	7.07	0.07	5.67	1.33

TABLE 2

Retentions of iron on an intake of approximately 10.4 mg a day for 4 weeks

SUBJECT	INTAKE	EXCRETION		RETENTION
		Urine	Feces	
	mg/day	*mg/day*	*mg/day*	*mg/day*
A	10.36	0.06	8.50	1.80
B	10.41	0.06	8.56	1.79
C	10.45	0.07	8.01	2.37
D	9.90	0.08	7.29	2.53
E	10.45	0.07	8.61	1.77

Mean losses of iron per menstrual period were calculated for each subject from the iron lost during the two or three menstrual periods occuring during the 10 weeks; they ranged from 10.94 to 20.14 mg (table 3). The mean length of the menstrual cycle for 4 of the subjects, during the experimental period and for a later consecutive three-month period, ranged from 26 to 35 days. From the mean cycle length and

mean loss per menstrual period, the amount of iron which would have to be retained every day in order to replace the losses was calculated. For 4 subjects the values were 0.50, 0.52, 0.58 and 0.62 mg per day; for the 5th subject, whose menstrual cycles were atypical, no calculation was made.

TABLE 3

Daily retention of iron required to replace menstrual losses

SUBJECT	IRON LOST IN MENSTRUAL PERIODS			MEAN LOSS PER PERIOD	MEAN CYCLE LENGTH	DAILY RETENTION TO COVER LOSS
	I	II	III			
	mg	*mg*	*mg*	*mg*	*days*	*mg*
A	16.15	18.16		17.15	34	0.50
B	17.02	17.78		17.40	28	0.62
C	13.97	16.43	10.27	13.59	26	0.52
D	21.06	10.27 [1]	28.08	20.14	35	0.58
E	10.94			10.94 [2]		

[1] Subject D reported short periods with small loss similar to period II about twice a year. Her mean loss per period calculated on a yearly basis would, therefore, be greater than 20.14 mg.

[2] In the case of subject E, only one period occurred during the study. Since the intervals between periods were atypical, the daily retentions required to cover her losses were not calculated.

DISCUSSION

Before deciding whether or not the retentions of the subjects were enough to cover their needs, possibilities of iron loss from the body other than in the menses must be taken into consideration. A complete collection of all the menstrual loss is almost impossible for every period even with the most meticulous care and with the use of the tampon-type pad. The iron content of the uncollected menses, however, falls within the experimental error. A small amount of iron is lost in hair and skin cells. In short, there are slight iron losses usually not accounted for in balance experiments, each loss small enough in itself to be within experimental error, yet when they are added together the total may be large enough to increase significantly the amount of iron which must be retained.

The adequacy of the 7-mg intake is questionable. The daily iron retentions (table 1) of subjects A, D and C were almost exactly the same as the amount needed to replace the loss in the menses (table 3); subject B retained less than was lost; and subject E retained so much that the retentions were almost sure to cover the losses although the losses were not calculated on a daily basis. If the accumulation of small losses in the uncollected menses, perspiration, hair and skin cells is taken into account, the retentions of these subjects, with the probable exception of subject E, were too near the borderline for safety.

The adequacy of the 10.4-mg intake cannot be questioned. The retentions were more than adequate to cover the menstrual and other small losses. The diet, however, was unusual in that it contained meat in every meal.

In order to assess the adequacy for women in the population as a whole of retentions of the sizes found on the two levels of intake used in this study, the most important factor to consider is the magnitude of the iron losses in the menses. After a literature survey, Frenchman and Johnston ('49) reported that the iron losses in the menses of 184 women had been investigated. The amount of iron needed every day of the year to replace the losses ranged from 0.08 to 2.6 mg (one abnormally high value was omitted). For 142 women the range was from 0.21 to 1.21 mg per day; that is, the requirement of iron to replace menstrual losses is 6 times as great for some women as for others. For 56% of the 184 women the mean daily retention of iron needed to replace the loss incurred during the few days of the menstrual period was more than ½ mg per day. Thus, the retention of ½ mg per day found on the 7-mg intake in the present study was not only too near the borderline for these subjects but it was, also, too small an amount to satisfy the needs of 56% of the 184 women studied by others. A retention high enough to cover the needs of 98 or 99% of all 184 women would be so high that extraordinary diets would have to be employed to supply it. To set that high a retention as necessary for all women would

be unwise, because most women would not need so much. A study of the frequency distribution of cases for each 0.1 mg of retention showed that beyond 1.2 mg the number of cases in each group was few. A retention of 1.2 mg, therefore, was taken as a practical amount to require for all women, although it covered the needs of only 86% of the 184 women studied. In the present study retentions on the 10.4-mg intake were large enough to more than satisfy such a requirement.

Women whose iron losses are great may absorb a higher percentage from food than other women and consequently not need a proportionately higher intake. That the "need" of the individual influences absorption to some degree under some conditions is probable, but no study has as yet been made to show whether or not large losses of iron in menses affect the percentage of absorption.

The possibility that the young women subjects of the present study might have been growing at a very slow rate and, therefore, absorbing more iron than older women must be considered. If a growth effect existed, however, it could not have been large. The possibility that these young women were absorbing more iron than men should be considered, also. Widdowson and McCance ('42) found an average absorption of 7.3% for 4 men and 12.5% for 4 women. The same authors ('48) demonstrated that female rats during the reproductive period stored more iron in their livers than male rats.

The question arises of whether or not the mean loss of approximately ½ mg of iron per day in the menses for the subjects of this study during the 10-week period would be similar to the mean for an extended period of a year or more. The review of the literature showed that the loss from period to period varies considerably for a given individual and that figures for at least 4, and possibly more, periods are necessary to arrive at an accurate evaluation of losses over an extended period of time. Since the mean losses for the subjects in the present study were based on only two or three menstrual periods, the values may not correspond exactly to

the mean for a year but could not be expected to vary unduly from that figure.

Several studies of iron retentions by women on intakes of 7 mg or less have been made. In some of them unusual combinations of foods were used and no retention of iron was found. On the other hand, in a study by Leverton ('41) in which the diet employed was unusual in that it contained large amounts of beef, the retentions were very large. Only one balance experiment at the 7-mg level has been carried out in recent years with women on diets customarily eaten by groups of the population. In that one (Leverton and Marsh, '42) the subjects were maintained on their usual self-chosen diets. The mean retention for 15 young women on an average intake of 7.16 mg per day was 0.41 mg, a retention similar to that found in this study.

Several investigations have been made recently with women on iron intakes in the neighborhood of 10 to 11 mg per day. Leverton and Roberts ('37), working with 4 subjects on customary diets containing 10.0, 10.7, 11.9, and 11.9 [2] mg of iron, found retentions of 0.71, —0.20, 1.48 and 1.07, respectively. Leverton and Marsh ('42) found that 31 subjects on an intake of 8.00 to 9.99 mg retained an average of 0.96 mg, and 31 other subjects on an intake of 10.00 to 11.99 mg retained an average of 1.34 mg. Widdowson and McCance ('42) reported that 4 subjects on an average intake of 12.5 mg retained 1.8 mg. The indication is that 10 to 11 mg of iron a day may be enough to obtain a retention of 1.2 mg. Such a retention might be expected to cover the needs of women living on diets customary in this country. That 10 to 11 mg of iron a day may be more than is needed on certain unusual diets was found in the present study, in which retentions were more than adequate when beef was included in every meal. On the other hand, 10 to 11 mg may be insufficient when certain other combinations of food are used: Widdowson and McCance ('42), with 4 women

[2] The average values for this subject are different from those reported by the authors because a period when ferric ammonium citrate was administered has been omitted.

on a diet containing 21.1 mg (far more than 10 to 11 mg) with 40 to 50% of the calories derived from brown bread, found an average retention of only 0.2 mg per day. More studies using typical diets with an iron content of 10 to 11 mg need to be made.

In the light of the results of this study and a review of the literature, the allowance of 12 mg of iron a day recommended by the National Research Council seems to be satisfactory for women on diets which do not contain large amounts of foods that inhibit iron absorption. That a few women may need more and many do not need that much, must be recognized. Further work may demonstrate that the allowance can safely be dropped a little.

SUMMARY

Five women 18 years of age were maintained for 10 weeks on a controlled diet. The total period was divided into a two-week adjustment period and two 4-week experimental periods. During the first experimental period the diet contained 7 mg of iron a day and during the second period sufficient beef was added to raise the intake to 10.4 mg.

On the intake containing approximately 7 mg of iron a day, retentions for the 5 subjects were 0.26, 0.43, 0.61, 0.64 and 1.33 mg per day. The amount of iron needed per day to replace menstrual losses for 4 subjects was 0.62, 0.52, 0.58 and 0.50 mg; no value is reported for the fourth subject because the menstrual cycles were atypical. The retentions for three subjects were approximately the same as the amount needed to replace menstrual losses, and for one subject were less than the amount needed. The retentions were too low for safety because even in the three cases where retentions corresponded to menstrual losses no margin existed to care for small losses not usually included in balance experiments.

On the intake of approximately 10.4 mg of iron, retentions for the 5 subjects were 1.77, 1.79, 1.80, 2.37 and 2.53 mg per day. These retentions were more than adequate to satisfy the needs of the subjects. It should be noted that these high

retentions were obtained on a diet containing beef in every meal.

LITERATURE CITED

FRENCHMAN, R., AND F. A. JOHNSTON 1949 The relation of menstrual losses to the iron requirement. J. Am. Diet. Assoc., *25:* 217.

JOHNSTON, F. A., R. FRENCHMAN AND E. D. BOROUGHS 1948 The absorption of iron from beef by women. J. Nutrition, *35:* 453.

LEVERTON, R. M. 1941 Iron metabolism in human subjects on daily intakes of less than five milligrams. Ibid., *21:* 617.

LEVERTON, R. M., AND A. G. MARSH 1942 The iron metabolism and requirement of young women. Ibid., *23:* 229.

LEVERTON, R. M., AND L. J. ROBERTS 1937 The iron metabolism of normal young women during consecutive menstrual cycles. Ibid., *13:* 65.

NATIONAL RESEARCH COUNCIL 1948 Recommended Dietary Allowances. Reprint and Circular Series No. 129.

STUGART, R. 1931 Determination of iron in milk and other biological materials. Ind. Eng. Chem., Anal. Ed., *3:* 390.

WIDDOWSON, E. M., AND R. A. MCCANCE 1942 Iron exchanges of adults on white and brown bread diets. Lancet, *1:* 588.

———— 1948 Sexual differences in the storage and metabolism of iron. Biochem. J., *42:* 37.

THE NUTRITIVE VALUE OF COD ROE AND COD LIVER

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ONE FIGURE

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During the months of February and March great shoals of cod visit the banks off the Norwegian coast for spawning, and the most extensive cod fishing of Norway takes place during this time.

The most important by-products of these fish are the cod liver and roe, which amount to several thousand tons a year each. The mean value of the catch from the years 1930–1944 was 13,000 tons of cod liver and 6,000 tons of cod roe, as reported by the Director of Norwegian Fisheries ('44).

The main part of the cod liver is used for the production of cod liver oil. A minor amount of the liver, however, has in recent years been used for the manufacture of canned pastes, often in combination with cod roe.

The roe is used in the fresh state as a valuable food by the people in coast regions. It is also canned. The main part, however, was formerly salted (cured) and exported to France and Portugal, where it was used as bait by the sardine fisheries.

The manufacture of canned cod liver-cod roe pastes has steadily increased in the last few years, and its product is now becoming of some importance for the Norwegian canning industry.

Cod liver has for a long time been the chief natural source of vitamins A and D as a supplement to common foods. Further investigations have shown that it also contains other vitamins and valuable proteins.

Investigations of cod roe reveal that it contains appreciable amounts of vitamins of the B group, proteins of high biological value, and also other substances of biological significance.

During the past few years much research work has been done in this institute to investigate the nutritive value of cod roe and cod liver. This paper summarizes previous studies and gives results of new investigations, especially concerning the biological value of the proteins.

The main constituents of the roe and liver are given in table 1, in terms of average values resulting from several analyses.

TABLE 1

The composition of cod roe and cod liver

CONSTITUENTS	COD ROE	COD LIVER
	%	%
Water	71.0	27.0
Protein	23.5	7.0
Fat	1.3	65.0
Phospholipids	2.6	1.0
Ash	1.5	

RESULTS AND DISCUSSION

The B vitamins of cod roe and cod liver

Thiamine (vitamin B_1). The thiamine content of fish and fish products has been determined by Lunde, Kringstad and Olsen ('38), biologically by the bradicardya method as well as chemically by the thiochrome method. The values vary considerably according to the condition of the roe. At the time of spawning the water content of the roe increases, and the effect seems to be that of dilution; the vitamin content calculated on the whole roe, however, is likely to remain fairly constant. The thiamine content of different samples of roe

in different seasons was found to range between 6 and 12 μg per gram. In several samples of fresh cod liver investigated, the thiamine content ranged between 2.5 and 3.5 μg per gram.

Riboflavin (vitamin B_2). Riboflavin is present in considerable amounts in cod roe and cod liver as compared with other foodstuffs. Determinations of this vitamin were carried out biologically by the growth method and by a chemical method worked out in this laboratory by Lunde, Kringstad and Olsen ('39). The latter is based upon measurement of the color, before and after reduction of the riboflavin to the leukobase, in the Pulfrich photometer using filter S45. Before a reading can be made, the extract must of course be subjected to several purification steps, including adsorption of the vitamin on Fuller's earth. The riboflavin content was determined in several samples and found to range between 6 and 15 μg per gram of roe. In fresh cod liver the riboflavin content varied from 5 μg to 10 μg per gram.

Nicotinic acid (niacin, P-P factor). This vitamin was determined chemically according to the method of Kringstad and Naess ('39). As compared with other sources, the cod roe and liver contain but small amounts of nicotinic acid (amide). Values between 10 and 25 μg per gram were found.

Pantothenic acid. In some samples of canned roe the pantothenic acid was determined microbiologically by the method of Kringstad and Folkvord ('45), using *Streptobacterium plantarum* as the test organism. The test was carried out according to the compensation method developed by Nielsen, Hartelius and Johansen ('43) and modified by Kringstad and Folkvord ('45). This method has the advantage of taking into consideration unknown factors in the test solution which might have an influence on the growth of the test organism.

P-aminobenzoic acid. Para-aminobenzoic acid was determined chemically according to the method of Schmidt and Kölbl ('44). The *p*-aminobenzoic acid is diazotized and coupled with α-napthol and the resulting color measured. Tests with known amounts of *p*-aminobenzoic acid showed fairly good results, the error not exceeding 7% of the added amounts.

The concentration of pantothenic acid and *p*-aminobenzoic acid in canned roe, including that which is dissolved in the added brine, is given in table 2 (Kringstad and Folkvord, '45).

Other vitamins of the B complex. Although no determinations of the other members of the B complex have been carried out, it is evident from animal experiments that cod roe and liver are good sources of these vitamins. This was reported by Lunde and Kringstad ('38) and Kringstad and Lunde ('40), who performed growth experiments on rats and foxes, which were placed on a synthetic diet deprived of all B vitamins and their growth restored when small amounts of cod roe extract were added. The same growth-restoring effect was

TABLE 2

The pantothenic acid and p-aminobenzoic acid content of cod roe

SAMPLE NO.	YEAR PRODUCED	D-PANTOTHENIC ACID PER 100 GM OF DRY MATTER	P-AMINOBENZOIC ACID PER 100 GM OF DRY MATTER
		mg	*mg*
1	1945	10.9	1.16
2	1945	12.6	1.02
3	1944	12.6	0.50
4	1940	12.6	0.47
5	1931	11.6	0.53

also obtained by these workers with the addition of defatted cod liver.

In experiments on foxes Ender and Helgebostad ('47) have recently found that on feeding stockfish a poor quality of fur resulted. By replacing some of the stockfish with cod roe, and to a minor extent with whole meal, a normal fur was developed. They ascribe this effect to a factor which protects the animals against greying (achromotrichia) and which is present in cod roe.

Vitamin C. Through chemical and biological analyses the presence of vitamin C in cod roe was determined by Mathiesen ('38). The values ranged between 0.2 and 0.4 mg per gram. When calculated on whole roe, the vitamin C content was found to be fairly constant, about 50 mg per gram.

The fat soluble vitamins of cod roe and cod liver

Vitamin A. The vitamin A content was investigated in some samples of fresh roe by the Carr-Price method. By saponification and isolation of the unsaponifiable matter, a greenish color developed when the $SbCl_3$-reagent was added to concentrated solutions in chloroform. The color seemed to be somewhat irregular as compared with that of pure vitamin A, and probably is due chiefly to other substances present. At any rate, the vitamin A content of roe must be regarded as negligible.

The mean value of vitamin A in cod liver found by spectrographical, biological and colorimetrical analyses is 600 I.U. per gram.

TABLE 3

The vitamin D content of fresh and canned cod roe and cod liver

SAMPLE NO.	MATERIAL STUDIED	VITAMIN D PER 100 GM
		I.U.
1	Fresh roe	85
2	Fresh roe	65
3	Canned roe	80
4	Canned roe	80
5	Fresh liver	6,000
6	Canned liver	6,000

Vitamin D. Our determinations of vitamin D were performed on representative samples of fresh and canned cod roe and liver by the usual X-ray method. The values found are given in table 3.

Vitamin E. Vitamin E was determined by the authors according to the method of Emmerie and Engel ('38, '39) as modified by Kjölhede ('43). A weighed sample of the homogenized roe was saponified in an atmosphere of nitrogen and then extracted by ethyl ether. After removal of the ether in a vacuum, the unsaponifiable matter was dissolved in petrol ether and filtered through a column of Fuller's earth to remove vitamin A and other substances which might interfere with the reagent.

The values for vitamin E calculated from these measurements were astonishingly high. When calculated on the basis of dry matter, cod roe contains nearly as much of this vitamin as the amount usually found in wheat germ meal.

As these results are based only on the chemical method of determination, they have to be verified by further experiments, especially through concentration by molecular distillation.

It is interesting in this connection to note that we have not found noticeable amounts of vitamin E in cod liver, nor in any other fish products except cod roe.

TABLE 4

The vitamin E content of fresh and canned cod roe

SAMPLE NO.	MATERIAL STUDIED	VITAMIN E PER 100 GM
		mg
1	Fresh roe	5.25
2	Fresh roe	7.60
3	Canned roe	5.85
4	Canned roe	7.70

The phospholipids of cod roe

The relatively high content of phospholipids found in cod roe is interesting, and we have therefore investigated this fraction to determine the content of the different phospholipids.

Extraction. Several methods of extracting the total phospholipids from the roe were investigated. The method of Schramme ('39) was found to be the most reliable and this method was therefore used.

The roe was ground with dry Na_2SO_4 and sand in a mortar and extracted with benzene-alcohol 80:20 on the waterbath in a Soxleth apparatus for 5 hours. The solution was evaporated to dryness in a vacuum and the phospholipids dissolved in dry ether.

Total phospholipids. This determination was carried out according to the method of Thaler and Just ('44).

An aliquot of the ether solution of the phospholipids was placed, together with 2.5 gm MgO *p.a.*, in a platinum crucible. The ether was evaporated on a water-bath and the organic matter decomposed by combustion with the MgO at 800°C.

After cooling, the content of the crucible was dissolved in HNO_3-H_2SO_4 and the phosphorus precipitated with ammonium-molybdate as usual. From the found phosphorus the phospholipids are calculated by the conversion factor 25.45, corresponding to a stearo-oleo-lecithin with molecular weight of 788 and containing 3.929% phosphorus.

Lecithin + sphingomyelin. The method used for this determination was that of Thannhauser, Benotti and Reinstein ('39). The choline, which is liberated by hydrolysis of the phospholipids with methyl alcoholic HCl, is precipitated by Reinecke salt. The precipitate is washed with chloroform, alcohol and ether and then dissolved in acetone. The resulting color is measured in the Zeiss Stepphotometer using the filter S53. From a calibration curve with known amounts of choline-reineckate the content of lecithin + sphingomyelin is found by multiplying by the factor 1.92.

TABLE 5

The phospholipids of cod roe

PHOSPHOLIPID	SAMPLE 1	SAMPLE 2
	%	%
Phospholipid content of roe	2.66	2.70
Sphingomyelin content of total phospholipids	42.6	45.2
Lecithin content of total phospholipids	45.6	45.0
Cephalin content of total phospholipids	11.8	9.8

Sphingomyelin. This determination was carried out according to the method of Thannhauser and Setz ('36), by precipitating the sphingomyelin as reineckate. The weight of sphingomyelin-reineckate multiplied by 0.788 gives the weight of sphingomyelin.

Cephalin [= *total phospholipids — (lecithin + sphingomyelin)*]. Table 5 gives the results of the phospholipid determinations of two samples of cod roe.

The digestibility and biological value of cod roe and cod liver proteins

The main constituent of cod roe is protein, which, on an average, amounts to 23.5% of the fresh roe, or 81% as calculated on the basis of dry matter. The eggs of the roe serve as a nutrient source for the developing organism, and it is therefore to be expected that the proteins have a high biological value and probably contain all essential amino acids. Although fat is the chief component of the cod liver and the protein only amounts to 7%, it is of importance to ascertain whether this protein is of any biological significance.

The method used in these investigations was essentially that of Mitchell ('24), with slight modifications. It has been shown by Boas Fixsen ('30) that vitamins of the B group are of importance in determining the biological value of proteins by animal experiments. Through lack of B factors, especially B_1, loss of appetite causes a decrease in food consumption, which results in a low caloric value for the food consumed. Hence the biological value of the proteins will be found to be too low.

In our experiments adult rats, with a weight of 170 to 230 gm for males and 160 to 185 gm for females, were used. The animals were placed in separate cages provided with a screen bottom, arranged on a funnel in such a way that feces and urine could be collected separately.

The composition of the basal nitrogen-free diet used is given in table 6. In addition, the animals received 20 μg thiamine, 20 μg riboflavin, 20 μg pyridoxine, 20 μg *p*-aminobenzoic acid, 100 μg nicotinic acid, and 300 μg Ca-pantothenate. These B factors were given orally (in water solution) from a calibrated pipette daily.

The nitrogen-free diet was fed for an 8-day period and feces and urine were collected for the last 5 days. In order to prevent losses of volatile nitrogen compounds during storage, due to decomposition, the feces were placed in concentrated sulphuric acid. To the urine-collecting flask a few

milliliters of 50% acetic acid were added, and the funnel and bottom screen of the cages were washed with diluted acetic acid. The urine was stored under toluene in a refrigerator.

During a following period of 8 days a diet contaning 8% of the protein to be tested was fed. The food was given ad libitum and the food consumed recorded each day. Feces and urine were collected as described above.

The total feces and urine excreted by each rat during the 5-day period were digested according to Kjeldahl, and finally diluted with water to a desired volume. Aliquots of these solutions were used for nitrogen determinations according to the semimicro-Kjeldahl method.

TABLE 6

Composition of nitrogen-free diet used

INGREDIENT	AMOUNT
	%
Potato starch	78.2
Sucrose	6.0
Arachis oil	8.0
Cod liver oil	2.0
Salt mixture (McCollum)	5.0
Calcium carbonate	0.8

The nitrogen content of the basal diet was negligible, and the nitrogen excreted during feeding on this diet is therefore assumed to be endogenous nitrogen.

Experiments were carried out on fresh, canned and dried cod roe, and on samples of defatted fresh and canned cod liver. For comparison, tests were also carried out on commercial casein.

The digestibility of the proteins was calculated according to the following formula:

$$D = 100. \frac{\text{N intake} - (\text{N in feces} - \text{N in feces endogenous})}{\text{N intake}}$$

The biological value was calculated according to Mitchell's formula, namely:

$$BV = 100. \frac{\text{N absorbed} - (\text{N in urine} - \text{N in urine endogenous})}{\text{N absorbed}}$$

N absorbed was calculated according to the expression:
N absorbed = N intake − (N in feces − N in feces endogenous).

It is obvious that the excretion of endogenous nitrogen is subject to variations which may influence the results to a noticeable degree. In order to get as reliable values as possible, both fecal and urinary endogenous nitrogen were determined for each animal before and after each test period.

In table 7 the digestibility and biological values of different samples of cod roe and of defatted fresh and canned cod liver are given. The results of a test on a commercial dried casein are also included.

TABLE 7

Digestibility and biological value of cod roe and cod liver proteins (The values are given as means of 10 rats.)

	FRESH ROE	CANNED ROE	DRIED ROE	FRESH LIVER	CANNED LIVER	CASEIN
Digestibility	81.3	80.8	79.2	68.7	70.5	84.4
Standard deviation of the means	1.26	1.35	0.78	1.19	1.39	1.22
Biological value	88.4	87.6	88.2	76.4	77.7	78.8
Standard deviation of the means	0.71	0.92	0.40	0.90	0.70	1.26

Growth experiments on rats with cod roe as protein source

In confirmation of the results obtained in the metabolic experiments, and especially to discover whether the proteins contain all essential amino acids, growth experiments with cod roe as the sole protein source were performed.

The composition of the basal diet was the same as that described above, except that the potato starch was replaced by purified rice starch. The ingredients were mixed with the test material to make a protein content of 14% as calculated on dry matter. No synthetic B vitamins were added in these experiments, since the roe has been shown to be a good source of growth factors of the B complex.

Rats with a weight of 50 to 60 gm, 4 males and 4 females, were used in each test. The diet was given ad libitum and the food consumed determined weekly. The experiment lasted for 7 weeks. In table 8 the values are given as means for two rats (placed in each cage) per week. As will be seen, the average weight increase for the animals on fresh cod roe was 118 gm for males and 102 gm for females. The corresponding values for rats on canned roe were 86 and 84, respectively. The rats in the last group did not take the food as well as those on the fresh roe, and the food consumption

TABLE 8

Growth experiment with fresh and canned cod roe
(Duration 7 weeks)

DIET	NUMBER OF RATS	SEX	TOTAL FOOD INTAKE	TOTAL PROTEIN INTAKE	TOTAL WEIGHT INCREASE	PROTEIN EFFICIENCY RATIO
			gm	*gm*	*gm*	
Fresh	4	♀	1,351	189	407	2.15
cod roe	4	♂	1,562	218	471	2.16
Canned	4	♀	1,171	164	336	2.05
cod roe	4	♂	1,210	169	344	2.03

of these animals was somewhat lower. The lesser weight increase of the animals on canned roe can therefore not be taken as an indication of the lack of some nutritive factor. When the weight increase is calculated per gram of protein consumed, the values for canned roe are only slightly lower than those for fresh, being 2.04 and 2.16, respectively.

Finally, an experiment with canned cod roe as the only nutrient source was carried out, 4 male rats with a weight of about 60 gm being fed canned cod roe for three weeks. The animals took the food well and showed excellent growth response during the whole period. The growth curves are shown in figure 1.

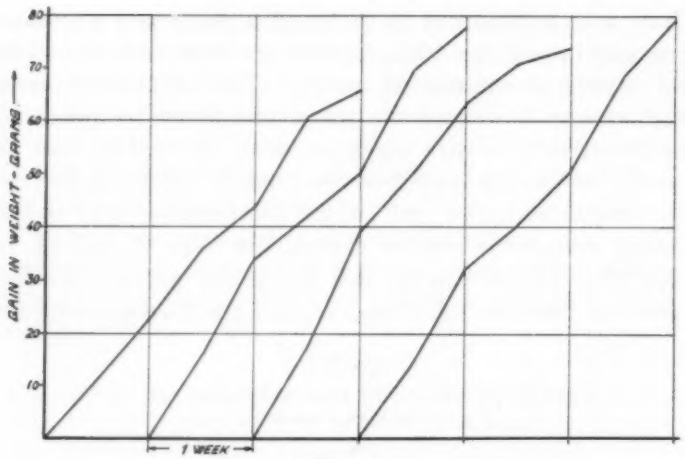

Figure 1

SUMMARY

In earlier studies it was found by extensive investigations that cod roe and cod liver are valuable sources of vitamins of the B complex. Cod roe especially was found to be rich in B vitamins, and to contain also appreciable amounts of vitamin C. The high content of vitamin A and D in cod liver is well known and has been appreciated for years. In the present paper new data from further investigations of the nutritive value of cod roe and cod liver are given.

Only traces — if any — of vitamin A were found (Carr-Price reaction) in cod roe. The vitamin D content amounted to about 80 I.U. on the average per 100 gm. The colorimetric determination, made according to the method of Emmerie and Engel ('38, '39), gave evidence of the presence of considerable amounts of vitamin E. Calculated on dry matter, the vitamin E content in roe is nearly as high as in wheat germ meal.

The phospholipid content of cod roe was found to be 2.70%, and the phospholipids shown to consist of 45% lecithin, 44% sphingomyelin and 11% cephalin.

Investigations of the nutritive value of cod roe proteins were carried out, and gave results of 81% digestibility and 88% biological value. No significant differences among fresh, canned and dried roe were found. For comparison, the same experiments were performed with casein. The results showed that the biological value of cod roe proteins is considerably higher than that of casein. The proteins of defatted fresh and canned cod liver were also investigated in the same way, and gave values of 69% for digestibility and 77% for biological value. No noticeable difference was found between fresh and canned cod liver with respect to digestibility and biological value of the proteins.

Growth experiments on rats fed cod roe proteins indicated the presence in the proteins of all essential amino acids.

CONCLUSIONS

Cod roe as well as cod liver has been shown to be a good source for substances of biological significance. Cod roe is valued chiefly owing to its high content of B vitamins and first-grade proteins. Cod liver is valued mainly because of its high content of fat, the fat soluble vitamins A and D, and B vitamins.

LITERATURE CITED

Boas Fixsen, M. A. 1930 The biological value of proteins. II. The biological value of purified caseinogen and the influence of vitamin B_2 upon biological values, determined by the balance sheet method. Biochem. J., *24:* 1794.

Director of Fisheries 1944 The Norwegian Fisheries. Norwegian Official Statistics, X 150.

Emmerie, A., and C. Engel 1938 Colorimetric determination of α-tocopherol (vitamin E). Rec. trav. chim., *57:* 1351.

———— 1939 Colorimetric determination of α-tocopherol (vitamin E). II. Adsorption experiments. Ibid., *58:* 283.

Ender, F., and A. Helgebostad 1947 The influence of the feeding on the quality of the fur of silver foxes. Norsk Veterinaertidskr., *12:* 381.

Kjölhede, K. T. 1943 On the chemical determinations of tocopherol. Nyt Nordisk Forlag, Arnold Busck, Köbenhavn.

Kringstad, H., and S. Folkvord 1945 Cod roe as a source of vitamin B. Tidsskr. f. Kjemi. Bergv. og Met., *5:* 104.

Kringstad, H., and G. Lunde 1940 The significance of the vitamin B-complex for silver foxes. Skand. Veterinaertidsskr., *21:* 1121.

KRINGSTAD, H., AND T. NAESS 1939 A colorimetric method for the estimation of nicotinic acid and its amide in foodstuffs. Zschr. physiol. Chem., *260:* 108.

LUNDE, G., AND H. KRINGSTAD 1938 Vitamin B-factors in roe and fish liver. Tidsskr. f. Hermetikkind., *24:* 184.

LUNDE, G., H. KRINGSTAD AND A. OLSEN 1938 The content of vitamins B_1 and B_2 of foods, especially of fish and fish products. Avh. Norske Vidensk.-Ak. Oslo 1. Mat.-Naturv. Klasse No. 7.

———— 1939 Estimation of lactoflavin (vitamin B_2) in natural products. Zschr. physiol. Chem., *260:* 141.

MATHIESEN, E. 1938 Vitamin C in cod roe. Tidsskr. f. Hermetikkind., *24:* 153.

MITCHELL, H. H. 1924 A method for the determination of the biological value of protein. J. Biol. Chem., *58:* 873.

NIELSEN, N., V. HARTELIUS AND G. JOHANSEN 1943 Simultaneous determination of pantothenic acid and β-alanine. Naturwiss., *31:* 550.

SCHMIDT, K. H., AND C. KÖLBL 1944 Separation and determination of *p*-aminobenzoic acid. Zschr. physiol. Chem., *281:* 7.

SCHRAMME, A. 1939 Determinations of phosphatides. Fette u. Seifen, *46:* 635.

THALER, H., AND E. JUST 1944 On the determination of phosphatides in fats. Ibid., *51:* 55.

THANNHAUSER, S. J., J. BENOTTI AND H. REINSTEIN 1939 Studies on animal lipids. XIV. The determination of lecithin, cephalin and sphingomyelin in body fluids and tissues, with analyses of normal human sera. J. Biol. Chem., *129:* 709.

THANNHAUSER, S. J., AND P. SETZ 1936 Studies on animal lipids. XII. A method for quantitative determination of diaminophosphatide in organs and fluids. Application to stromata of red blood cells and serum. Ibid., *116:* 533.

CONCENTRATIONS OF VARIOUS CONSTITUENTS IN BLOOD OF DAIRY COWS DURING STAGES OF TERMINAL GESTATION AND INITIAL LACTATION

I. EFFECT OF PREPARTAL DIET ON SERUM TOCOPHEROLS [1]

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The importance of tocopherols (vitamin E) in animal nutrition suggested a study of this nutriment in the parturient dairy cow. Although considerable information on the function of this vitamin in laboratory animals is available, similar reports on dairy cattle are few. Recent studies have indicated that deficiencies of tocopherols may result in cardiac disorders (Gullickson and Calverley, '46). According to Harris et al. ('47), dietary supplementation of a typical herd ration with tocopherols during lactation improved the general health of cows and increased the fat content of milk and yield of "4% milk," but these observations have not been substantiated (Fountaine and Parrish, '48; Gullickson et al., '48; Phillips et al., '48; Whiting et al., '49). Supplementation of

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rations of dairy cows during the terminal stages of gestation augmented the secretion of tocopherols in colostrum and early milk (Parrish et al., '47).

The investigation reported herein was designed primarily to gain additional information on the role of tocopherols in dairy cattle by determinations of changes in the concentration of this vitamin in the blood serum during stages of terminal gestation and initial lactation of animals fed typical barn rations, unsupplemented or supplemented.

PROCEDURES

Grouping, feeding and management of experimental animals

Pertinent facts concerning the grouping of the experimental heifers and cows and the duration and levels of vitamin supplementation for each group are presented in table 1. Vitamin A supplementation during the two weeks preceding the scheduled date of parturition was doubled, since investigations of Sutton et al. ('45) revealed a marked tendency for concentrations of this vitamin in blood to decrease during this stage of pregnancy. When tocopherols were administered with vitamin A, the ratio of the two vitamins was uniform throughout the trial. All tocopherols used were in the free, unesterified form. Further details of the feeding and management of the experimental animals have been reported by Parrish et al. ('47).

Collection and preservation of blood samples

Samples of venous blood for analysis were obtained from the various groups of cows at the prepartal stages indicated in table 2 and from all groups immediately after parturition (0–6 hours) and also one, two, three, 7, 14, 27 and 28 days later. Collections were made from 14 to 16 hours following daily administration of vitamin supplements. After the blood had clotted, serum was obtained by centrifugation. When immediate analysis was not possible, the serum was stored in

tightly closed bottles protected from light and refrigerated at 4° C.

Analytical procedures

The analytical method used was the Quaife and Biehler ('45) modification of the Quaife and Harris ('44) procedure, except as follows: Removal of Skelly-solve B to obtain the lipid residue containing tocopherols was accomplished by

TABLE 1

Schedule of daily supplementation of diets of pregnant heifers and cows
(The $\alpha-\gamma$ tocopherols were either 50 or 90% γ form)

GROUP	SUB-GROUP	NUMBER OF ANIMALS	DAYS PREVIOUS TO DATE OF EXPECTED PARTURITION	
			28 — 14	13 — 0 [1]
I	a	5	No supplement	No supplement
	b	8	500,000 I.U. vit. A alcohol	1,000,000 I.U. vit. A alcohol
	c	6	500,000 I.U. vit. A ester	1,000,000 I.U. vit. A ester
II	–	6	500,000 I.U. vit. A alcohol and 0.5 gm $\alpha-\gamma$ tocopherol	1,000,000 I.U. vit. A alcohol and 1 gm $\alpha-\gamma$ tocopherol
III	a	3	10 gm mixed tocopherol	10 gm mixed tocopherol
	b	1	0.5 gm $\alpha-\gamma$ tocopherol	1 gm $\alpha-\gamma$ tocopherol
	c	1	4 gm mixed tocopherol	4 gm mixed tocopherol
	d	1	5 gm $\alpha-\gamma$ tocopherol	5 gm $\alpha-\gamma$ tocopherol
VI [2]	–	1	No supplement	No supplement

[1] Vitamin supplementation discontinued at parturition.
[2] Cow milked through gestation, without a dry period.

evaporation under suction, using a water bath at 50° to 60°C. for heating. The hydrogenated extract was warmed in a water bath at 35°C. prior to the addition of the analytical reagents. A Coleman spectrophotometer with a special cell holder that accommodated $7'' \times \frac{7}{8}''$ matched absorption tubes was used in the assay. Photometric readings were made at a wavelength setting of 520 mμ. The instrument was calibrated using pure natural α-tocopherol.[4]

[4] Supplied by M. L. Quaife, Distillation Products, Inc., Rochester, N. Y.

Method of adjusting analytical data

Attempts were made to obtain prepartal blood samples at intervals that would represent not only definite periods of vitamin supplementation (table 1) but also specific stages in the terminal stages of gestation (table 2), both of which affect tocopherol concentration in the serum. Since parturition frequently did not occur on the anticipated date, in several instances the first 4 prepartal samples collected according to the outlined schedule of supplementation did not correspond

TABLE 2

Prepartal stages at which blood samples were scheduled to be collected from experimental cows, compared with times of actual collection

SCHEDULED COLLECTION	ACTUAL COLLECTION (Averages and ranges)			
	Group I	Group II	Group III	Group IV
35 [1]	38 (51–25)	44 (58–35)	42 (49–35)	..
28 [2]	31 (41–18)	32 (50–21)	34 (37–28)	28
14 [3]	16 (27–9)	19 (38–8)	18 (26–14)	14
7	8 (11–6)	8 (14–5)	8 (11–7)	7
3	3	3	3	3
1	1	1	1	1

[1] Start of trials, a pre-supplementation period.

[2] Immediately prior to start of supplementation of cows that received vitamins.

[3] Immediately prior to increase of supplementation of cows that had levels of vitamin intake changed.

to the expected gestational stage. When this occurred, adjustments were made in the data from individual cows so that averages could be computed on a period basis. Interpolations from graphs representing the data from the cows were employed in those cases in which the time of blood collection did not correspond to the scheduled periods. The data, actual or interpolated when necessary, were averaged by dietary groups (tables 3 and 4).

When the data were examined with respect to breed and number of lactation periods, no consistent differences attributable to either of these two factors were found; hence they were disregarded in the computation of means.

RESULTS

Trends of tocopherol levels in blood serum of cows that received no tocopherol supplements

Cows directly off pasture frequently had high levels of blood serum tocopherols. Typical of these high values, expressed as μg per 100 ml, are 1,473, 898, 873, 737 and 724. The levels of serum tocopherols for other cows in corresponding stages of pregnancy that received only barn rations for the preceding two to 8 weeks were never more than 450 μg.

As parturition approached, a downward trend was exhibited in the tocopherol concentrations in blood serum of cows receiving no supplemental tocopherols (group I, table 3), the rate of decrease generally becoming more pronounced during the days immediately before parturition. The minimum values usually occurred on the second or third day *post partum,* after which the concentrations gradually increased (group I, table 4). At the end of the first month of lactation, the average levels were higher than at the initial period one month before parturition.

Comparisons of data from groups I-a, -b and -c indicate that supplementation of the prepartal diet with vitamin A had no significant effect upon either the magnitude of tocopherol concentrations or their trends in the blood serum.

The cow of group IV received a diet similar to that of the cows in group I-a (table 1) but was milked throughout gestation without the usual dry period. The levels of tocopherols in her blood serum, in contrast to those of other cows, increased slightly as gestation approached termination (table 3). The maximum concentration was observed on the first day *post partum* and the minimum on the 7th day (table 4).

Trends of tocopherol levels in blood serum of cows that received tocopherol supplements

Tocopherol levels of blood serum of preparturient cows that received vitamin A alcohol and 0.5 to 1 gm of tocopherol supplements daily (group II, table 3) were more than 15% higher

TABLE 3

Prepartal changes in tocopherol concentrations of blood serum from dairy cows subjected to various dietary treatments

GROUP	SUB-GROUP	DAILY SUPPLEMENT		STAGES (DAYS PREPARTAL) OF BLOOD COLLECTION					
				Pre-suppl.[1]		Initial suppl.	Terminal supplementation		
				35[2]	28	14	7	3	1
				Tocopherols, μg/100 ml					
I	a	None	*Av.*	....	433 (3)[3]	340 (5)	347 (5)	328 (5)	290 (5)
			Range	....	351–557	302–405	325–373	306–352	266–353
	b	Vitamin A alcohol	*Av.*	588 (5)	508 (6)	402 (6)	365 (7)	298 (8)	286 (8)
			Range	371–898	396–755	266–588	286–427	204–384	180–371
	c	Vitamin A ester	*Av.*	384 (3)	530 (5)	395 (6)	320 (6)	268 (6)	248 (6)
			Range	266–470	279–873	266–526	237–483	167–483	176–450
			Group mean	511 (8)	499 (14)	381 (17)	345 (18)	296 (19)	275 (19)
II		Vitamin A alcohol and 0.5–1 gm tocopherol	*Av.*	690 (5)	535 (6)	466 (6)	414 (6)	391 (6)	388 (6)
			Range	310–1,473	390–792	359–532	301–518	328–510	307–545
III	a	10 gm mixed tocopherol	*Av.*	351 (3)	479 (3)	1,268 (3)	1,333 (3)	1,201 (3)	1,164 (3)
			Range	198–427	297–724	890–1,764	930–1,836	965–1,594	919–1,622
	b[4]	0.5–1 gm tocopherol		384	396	569	559	557	378
	c[4]	4 gm tocopherol		724	588	1,187	1,278	1,271	1,136
	d[4]	5 gm tocopherol		619	378	919	861	805	813
IV[4,5]		None		...	283	341	353	353	365

[1] See table 1.
[2] See table 2.
[3] Number of samples represented in the means enclosed in parentheses.
[4] Only one cow in the classification.
[5] Milked through entire gestation.

TABLE 4

Postpartal changes in the tocopherol concentrations of blood serum from dairy cows receiving various prepartal dietary treatments

GROUP	SUB-GROUP	DAILY PREPARTAL SUPPLEMENT [1]		STAGES (DAYS) POST PARTUM							
				0 [2]	1	2	3	7	14	21	28
				Tocopherols, μg/100 ml							
I	a	None	*Av.*	276 (5) [3]	260 (5)	240 (5)	261 (5)	305 (5)	410 (5)	420 (5)	575 (5)
			Range	254–316	210–297	192–285	198–310	186–458	298–613	322–463	490–631
	b	Vitamin A alcohol	*Av.*	271 (8)	248 (8)	227 (8)	236 (8)	329 (8)	454 (2)	528 (2)	..
			Range	155–371	136–340	142–291	99–365	204–508	314–593	424–632	
	c	Vitamin A ester	*Av.*	234 (6)	233 (6)	213 (6)	228 (6)	275 (6)	..	..	..
			Range	115–433	149–384	155–340	155–340	192–470			
			Group mean	260 (19)	244 (19)	226 (19)	240 (19)	306 (19)	423 (7)	451 (7)	575 (5)
II		Vitamin A alcohol and 0.5–1 gm tocopherol	*Av.*	350 (6)	292 (6)	255 (6)	279 (6)	323 (6)	..	..	..
			Range	285–508	229–390	192–297	210–340	266–458			
III	a	10 gm mixed tocopherol	*Av.*	1,011 (3)	1,044 (3)	1,007 (3)	852 (3)	593 (3)	582 (3)	643 (3)	659 (3)
			Range	848–1,281	848–1,269	904–1,151	563–1,003	303–899	378–885	452–900	435–896
	b [4]	0.5–1 gm		396	279	347	285	369	501	513	616
	c [4]	4 gm tocopherol		1,003	891	848	823	836	799	799	842
	d [4]	5 gm tocopherol		817	650	582	557	396	594	680	799
IV [4,5]		None		347	396	359	316	241	379	..	..

[1] All supplementation discontinued at parturition.
[2] First sample after parturition.
[3] Number of samples represented in the means enclosed in parentheses.
[4] Only one cow in each classification.
[5] Milked throughout gestation.

after 14 days of supplementation than those of animals that did not receive additional tocopherols (group I). The high values in groups I-b and II at the initial periods possibly were due to residual effects of tocopherols obtained from pasture (Cabell and Ellis, '42), from which several cows were removed immediately before collection of first blood samples. The general prepartal trends of the tocopherol levels of groups I and II were similar, but the accelerated rate of decrease for group II began later, at the time of parturition. Concentrations of tocopherols in blood serum from group II immediately after parturition (table 4) remained higher than those of the controls (group I), but after 7 days of lactation the levels were approximately the same.

The addition of from 4 to 10 gm of tocopherols to the rations of animals in group III (table 3) increased markedly the levels of this vitamin in the blood serum. At high levels of intake, maximum levels of serum tocopherols generally were reached one to two weeks before parturition. Even though the rate of increase of tocopherols in the blood serum of the cow of group III-d was greater than that of the cow of group III-c, the latter consistently maintained a higher concentration throughout the trial. When 0.5 gm of tocopherol was introduced into the ration of the cow of group III-b, the concentration of this vitamin increased in the blood serum, but a similar amount of tocopherols given to cows in group II did not prevent the downward trend. Failure of supplementation to increase tocopherol content in serum from cows of group II probably was due to higher pre-supplementation levels.

At stages of supplementation 13 days to one day before parturition, concentrations of tocopherols in the blood serum of cows receiving 10 gm of this vitamin daily (group III-a) were approximately 4 times higher than those of the controls (group I). However, even this high level of supplementation did not prevent the downward trend of tocopherol concentrations as parturition approached.

The residual effects of prepartal tocopherol supplementation on postpartal concentrations of this vitamin in the blood serum seemed to be related to the levels of intake. When daily additions to the diet were 0.5 to 1 gm, and 10 gm, the minima occurred, respectively, from one to two days and from 7 to 14 days postpartally. After the termination of the decline in the control cows (group I), the differences between the tocopherol levels in the serum of these animals and of those receiving tocopherol supplements before parturition gradually diminished. Four weeks after calving, blood serum tocopherol levels of cows of group III-a were only about 15% higher than those of group I, and were within the same range as those of cows in groups III-b, -c and -d.

The various levels of vitamin supplementation produced no noticeable advantage in either the thriftiness or the reproductive health of the cow; there was no apparent effect on the incidence of retention of placental membranes.

DISCUSSION

Declines in concentrations of tocopherols in the blood serum of dairy cattle during the stages of terminal gestation and early lactation apparently are characteristic for cows subjected to conventional feeding and management practices. The trends are similar to those observed for vitamin A and carotene (Sutton et al., '45; Wise et al., '47). Diversity in the reported values of tocopherols in the blood of pregnant women suggests that the changes in human subjects do not follow so definite a pattern as has been noted for cattle (Straumfjord and Quaife, '46; Varangot, '42; Varangot et al., '43). It is improbable, however, that the parturient decreases in the concentrations of vitamin E in the blood of cattle are unique for this species.

The specific factors involved in the reduction of tocopherol levels have not been determined. Deficiency of the vitamin obviously was not an etiological factor at the levels of intake investigated, inasmuch as the decreases occurred regardless of dietary supplementation. Withdrawal of tocopherols from

the blood in the fortification of the early mammary secretions (Parrish et al., '47) possibly played a role in the reduction, but similar declines in the serum of a mammectomized cow receiving normal rations (Fountaine and Parrish, '48) indicated that other metabolic changes associated with parturition and initial lactation probably were involved to a greater extent than mammary functions *per se*.

Present information on factors affecting concentrations of tocopherols in blood is inadequate to explain the causes of some of the observed differences. In contrast to responses observed in other experimental subjects, the upward prepartal trend in the serum of the cow of group IV introduces the question of whether omission of the normal dry period affected tocopherol concentrations in the blood. Further investigation is needed to ascertain if the reaction was an individual peculiarity or is a typical response of cows that continue milk production throughout gestation.

The prepartal level of tocopherol intake was one of the primary factors affecting concentrations of this vitamin in the blood of the experimental subjects. The dietary responses were in accord with those noted in human beings (Couperus, '43; Quaife and Harris, '44; Wechsler et al., '41, '43), rabbits (Minot, '44), calves (Latschar, '47) and lactating cows (Fountaine and Parrish, '48; Harris et al., '47; Whiting et al., '49). A report by Kaay ('47) revealed further that the tocopherol contents of blood from cows, pregnant and non-pregnant, varied markedly according to the feed supplied. His results, in harmony with values observed in the first samples of blood serum from cows used in the current study, indicated that pasture grazing greatly increases the tocopherol levels. According to calculations of vitamin E intake, cows fed a typical barn ration consume approximately 1 gm daily (Phillips et al., '48), whereas cows eating 100 lb. of grass daily ingest 7.1 gm (Cabell and Ellis, '42). Therefore, the levels of tocopherols in the blood of well-fed cows would not be expected to change to any marked degree when small amounts of tocopherols are added to the ration.

Tocopherol supplementation during the terminal weeks of gestation affected levels and trends of this vitamin in the blood of cows not only prepartally but also postpartally. These residual effects indicated that the additional tocopherol intake augmented the body reserves.

The time of collecting the blood samples in relation to the ingestion of supplemental tocopherols may have been another factor related to the concentrations of this vitamin detected in blood serum. Couperus ('43) and Quaife and Harris ('44) reported that maximum concentrations of tocopherols in human blood were attained 6 hours after oral administration of a single dose. The time required for the levels to return to normal seemed to vary with the amount ingested. It is probable that the rate of absorption in ruminants is slower and more uniform than in monogastric animals. Though in the present study blood samples were drawn at a specific time, 14 to 16 hours after feeding the tocopherols, the stage represented in the absorption cycle was not ascertained.

In harmony with the report of Kaay ('47), the well-being of heifers and cows and their reproductive performance were unrelated to levels of tocopherol in the diet and in the blood. Thus barn rations seemingly provided sufficient amounts of this vitamin to meet minimum needs for normal completion of gestation and initiation of lactation. Since the present investigation covered only a small segment of the gestation-lactation cycle, longer periods of supplementation might have yielded different results; but the findings of Gullickson et al. ('44) using vitamin E-low rations indicate that this is improbable. Nevertheless, a more comprehensive exploration is warranted.

SUMMARY

1. Determinations were made of tocopherol concentrations in the blood serum of dairy cows receiving typical barn rations, unsupplemented or supplemented, during stages of terminal gestation and initial lactation.

2. Tocopherol concentrations in the blood serum of dairy cows restricted to typical barn rations decreased slowly dur-

ing the terminal month of pregnancy. The decline general'y became more pronounced within a few days before parturition and reached a minimum level the second day *post partum*, after which a gradual but continuous rise ensued.

3. Addition of vitamin A supplements to the diet of the preparturient dairy cow had no significant effect on tocopherol levels in the blood serum.

4. Addition of tocopherols (0.5 to 1 gm) to the diet resulted in concentrations of blood serum tocopherols averaging more than 15% higher than those in the controls.

5. Addition of large tocopherol supplements daily to typical barn rations of dairy cows during the concluding month of gestation resulted in an increase in tocopherol levels in blood serum, the highest level of tocopherol supplementation (10 gm daily) eliciting the greatest response (approximately 4 times higher than in the controls).

6. The higher levels of tocopherol supplementation, 4 gm to 10 gm daily, counteracted the gradual downward prepartal trend in blood serum concentrations of this vitamin which was characteristic of serum from cows receiving no tocopherol supplements, but did not prevent the rapid decline in the immediate parturient period.

7. After cessation of tocopherol supplementation at parturition, the *post partum* trends of blood serum tocopherols were similar for all groups; however, high levels of prepartal supplementation delayed the time of attainment of the minimum level and tended to maintain higher concentrations subsequently.

ACKNOWLEDGMENTS

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LITERATURE CITED

CABELL, C. A., AND N. R. ELLIS 1942 Vitamin E content of certain varieties of wheat, corn, grasses and legumes as determined by rat assay. J. Nutrition, *23:* 633.

COUPERUS, J. 1943 Untersuchung über die Bedeutung des Vitamins E in der Neurologie. Zschr. Vitaminforsch., *13:* 193.

FOUNTAINE, F. C., AND D. B. PARRISH 1948 Unpublished data.

GULLICKSON, T. W., AND C. E. CALVERLEY 1946 Cardiac failure in cattle on vitamin E-free rations as revealed by electrocardiograms. Science, *104:* 312.

GULLICKSON, T. W., J. B. FITCH AND L. D. GILMORE 1948 Effect of feeding tocopherols to dairy cows on the quantity and the fat content of milk produced. J. Dairy Sci., *31:* 557.

GULLICKSON, T. W., L. S. PALMER, W. L. BOYD AND F. C. OLSON 1944 Vitamin E in the nutrition of cattle. Ibid., *27:* 634.

HARRIS, P. L., W. J. SWANSON AND K. C. D. HICKMAN 1947 Covitamin studies. VI. Effect of tocopherol supplementation on the output of vitamin A, carotene and fat by dairy cows. J. Nutrition, *33:* 411.

KAAY, v. d., F. C. 1947 Vitamine E en steriliteit bij paard en rund. Tijdschr. Diergenessk., *72:* 571.

LATSCHAR, C. E. 1947 Changes in the concentration of tocopherols in the blood serum of the parturient dairy cow and her neonatal calf. Thesis, Kansas State College, Manhattan.

MINOT, A. S. 1944 The determination of tocopherol in blood serum. J. Lab. Clin. Med., *29:* 772.

PARRISH, D. B., G. H. WISE AND J. S. HUGHES 1947 Properties of the colostrum of the dairy cow. I. Tocopherol levels in the colostrum and in the early milk. J. Dairy Sci., *30:* 849.

PHILLIPS, P. H., J. KASTELIC AND E. B. HART 1948 The effect of mixed tocopherols on milk and buterfat production of the dairy cow. J. Nutrition, *36:* 695.

QUAIFE, M. L., AND R. BIEHLER 1945 A simplified hydrogenation technique for the determination of blood plasma tocopherols. J. Biol. Chem., *159:* 663.

QUAIFE, M. L., AND P. L. HARRIS 1944 Chemical estimation of tocopherols in blood plasma. Ibid., *156:* 499.

STRAUMFJORD, J. V., AND M. L. QUAIFE 1946 Vitamin E levels in maternal and fetal blood plasma. Proc. Soc. Exp. Biol. Med., *61:* 369.

SUTTON, T. S., H. E. KAESER AND P. A. SOLDNER 1945 Changes in the level of vitamin A and carotene in the blood plasma of dairy cows associated with parturition and beginning lactation. J. Dairy Sci., *28:* 933.

VARANGOT, J. 1942 Sur la teneur du sérum saguin en vitamin E au cours de la gestation humaine. Compt. rend. Acad. Sci., *214:* 691.

VARANGOT, J., H. CHAILLEY ET N. RIEUX 1943 Sur la teneur du sérum sanguin en vitamin E au cours de la grossesse et dans le post-partum. Compt. rend. Soc. biol., *137:* 393.

WECHSLER, I. S., G. G. MAYER AND H. SOBOTKA 1941 Tocopherol level in serum of normals and patients with amyotrophic lateral sclerosis. Proc. Soc. Exp. Biol. Med., *47:* 152.

———— 1943 Tocopherol level in human serum during oral tocopherol therapy. Ibid., *53:* 170.

WHITING, F., J. K. LOOSLI, V. D. KRUKOVSKY AND K. L. TURK 1949 The influence of tocopherols and cod-liver oil on milk and fat production. J. Dairy Sci., *32:* 113.

WISE, G. H., F. W. ATKESON, M. J. CALDWELL, D. B. PARRISH AND J. S. HUGHES 1947 Effects of high vitamin A intake on milk and fat yields and on vitamin A constituents in milk, blood, and livers of dairy cows. Ibid., *30:* 279.

NET PROTEIN AND GROWTH-PROMOTING VALUES OF THREE DIFFERENT TYPES OF YEAST PREPARED UNDER IDENTICAL CONDITIONS

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ONE FIGURE

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A previous investigation (Goyco and Asenjo, '46) has indicated that though the nitrogen content and true digestibility of different samples of yeasts were quite similar, there was marked difference between the biological values of strains of *Torula* and brewers' yeasts and, consequently, between their respective net protein values. Two different strains of *Torula utilis* exhibited biological values which were over 21% below those observed in a sample of brewers' yeast studied at the same time. This difference in biological values could have been due to the influence of the growing mash or to the processing used in their elaboration, since they were prepared with different nutrient mediums and at different places.

The purpose of the following two experiments was to study the protein quality of different species and varieties of yeasts which had been prepared under identical conditions.[1]

[1] Pure cultures of *Torula utilis* No. 1084, *Saccharomyces cerevisiae* ("Gebruder Meyer") No. 52 (Brewers' Yeast No. 52), and Fleischmann's Stock Bakers' Yeast No. 51 (Bakers' Yeast No. 51) were furnished by us to Mr. Carlos Vincenty, of the Puerto Rico Industrial Development Company, who prepared a quantity of each one of these yeasts by identical techniques. The growing mash used in their elaboration was Puerto Rican blackstrap molasses clarified and diluted to 5° Brix, with three grams of ammonium sulfate and 2 gm of ammonium acid phosphate (secondary) added per liter of the diluted molasses.

The writers are most grateful to Mr. Vincenty for his valuable cooperation.

EXPERIMENTAL

Determination of digestibility, biological, and net protein values

In order to ascertain the digestibility, biological, and net protein values of the proteins of the different yeasts, the technique recommended by Mitchell and Carman ('24) was used with some modifications. As the details of this procedure have been already described in a previous communication (Goyco and Asenjo, '46), they will not be repeated here.

The tests lasted for 10 days and were divided into two periods: a three-day preliminary period when no samples were collected, and a 7-day experimental period during which urine and feces samples were collected daily. All diets used in the metabolism experiments contained about 8% protein on a wet basis (N × 6.25). Their composition is given in table 1.

TABLE 1

Composition of diets

CONSTITUENTS [1]	STANDARDIZING DIET	TORULA UTILIS NO. 1084	BREWERS' YEAST NO. 52	BAKERS' YEAST NO. 51	TORULA UTILIS NO. 1084 AND 0.5% DL-METHIONINE
	%	%	%	%	%
Dried, ether-extracted whole egg	6.50	....	....	....	....
Dried yeast	....	14.45	16.80	16.95	14.45
Modified Osborne and Mendel salts	3.00	3.00	3.00	3.00	3.00
Sucrose	10.00	10.00	10.00	10.00	10.00
Sodium chloride	1.00	1.00	1.00	1.00	1.00
Vegetable oil (Mazola)	9.00	9.00	9.00	9.00	9.00
Cod liver oil	1.00	1.00	1.00	1.00	1.00
Corn starch	66.50	58.55	56.20	56.05	58.05
Cellu-flour	3.00	3.00	3.00	3.00	3.00
DL-Methionine	....	....	....	....	0.50
Protein content	4.44	7.62	8.06	8.12	7.66

[1] One drop daily of B complex concentrate was fed to each animal: 200 μg of thiamine; 20 μg of riboflavin; 2.25 μg of pyridoxine; 6.2 μg of pantothenic acid; 500 μg of niacin-niacinamide, and 0.4 units of filtrate factor.

The results of the initial and final standardizing periods are shown in table 2. These two values, when plotted, permitted the interpolation, during the testing period, of a specific value for each animal under observation.

From the nitrogen metabolism data assembled in table 3 the coefficients of true digestibility and biological and net protein values were computed in the usual way. The values thus obtained are reported in table 4.

TABLE 2

Daily excretion of metabolic and endogenous nitrogen during initial and final standardizing periods

	BODY WEIGHT		FOOD INTAKE	FECAL NITROGEN	METABOLIC NITROGEN IN FECES PER GRAM OF FOOD	URINARY NITROGEN	ENDOGENOUS NITROGEN IN URINE PER 100 GM WEIGHT
	Initial	Final					
	gm	*gm*	*gm*	*mg*	*mg*	*mg*	*mg*
Initial period: dried egg protein ration containing 0.71% N							
Average of 10 rats	55.4	67.1	6.5	7.7	1.2	12.7	20.8
Standard deviation	3.4	4.6	0.7	0.9	0.1	1.2	0.9
Final period: dried egg protein ration containing 0.71% N							
Average of 10 rats	103.3	110.0	7.7	10.2	1.3	14.9	14.0
Standard deviation	11.9	11.1	0.7	1.2	0.1	1.7	0.7

Although its digestibility was slightly higher, *Torula utilis* exhibited biological and net protein values much lower than either one of the *S. cerevisiae* strains studied. However, when 0.5% DL-methionine was added to the *Torula utilis* ration the coefficient of digestibility as well as the biological and net protein values increased to the very high figures of 90.2, 88.3, and 44.1%, respectively.

In the case of the two strains of *S. cerevisiae,* Brewers' No. 52 and Bakers' No. 51, the coefficient of digestibility and the biological and net protein values were almost identical in each case, 79.9 and 80.7, 58.4 and 58.9, and 22.2 and 22.7, re-

TABLE 3

Nitrogen metabolism of rats on yeast rations
(Results expressed on daily basis)

	BODY WEIGHT		FOOD INTAKE	NITROGEN			
	Initial	Final		Intake	In feces	In urine	Balance
	gm	*gm*	*gm*	*mg*	*mg*	*mg*	*mg*
			Torula utilis No. 1084 ration				
Average of 10 rats	60.1	58.1	2.49	30.4	7.6	29.0	— 6.2
Standard deviation	4.5	4.2	0.6	7.3	1.8	3.6	3.7
			Brewers' yeast No. 52 ration				
Average of 10 rats	60.1	66.0	4.70	60.6	17.9	31.6	11.1
Standard deviation	4.4	6.0	0.8	10.6	3.2	3.7	7.3
			Bakers' yeast No. 51 ration				
Average of 10 rats	68.7	74.3	4.62	60.1	17.4	31.8	10.9
Standard deviation	7.1	9.7	1.0	13.6	3.8	4.9	6.1
			Torula utilis No. 1084 and 0.5% DL-methionine ration				
Average of 10 rats	81.4	95.8	6.28	76.6	15.5	21.6	39.5
Standard deviation	11.1	12.3	1.0	12.4	2.2	2.6	8.0

spectively These values were lower than those previously reported by us (Goyco and Asenjo, '46) for a different strain of *S. cerevisiae.*[2]

Growth-promoting studies

For the growth studies, 28-day-old male Wistar albino rats, weighing 54 to 57 gm, were transferred from the stock colony to round double-bottom cages, three to a cage.

[2] Fleischmann Type 2019. No definite information could be obtained on the composition of the mash used to grow this yeast; probably it was a cereal mash.

Experimental yeast diets were fed to them ad libitum, the composition of these diets being the same as of those used in the previous experiment except for the percentage of yeast, which was increased to about 18% protein (table 5), and the percentage of cornstarch, which was reduced proportionally. A diet containing purified casein [3] as the sole source of protein was used for comparison. All animals were supplemented

TABLE 4

Coefficient of true digestibility, biological value and net protein value of the nitrogenous substances present in yeast

	TORULA UTILIS NO. 1084	BREWERS' YEAST NO. 52	BAKERS' YEAST NO. 51	TORULA UTILIS NO. 1084 AND 0.5% DL-METHIONINE
(a) % Protein (N × 6.25) in the yeast samples	55.37	47.68	47.25	55.37
(b) Total fecal nitrogen [1]	7.6	17.9	17.4	15.5
(c) "Metabolic nitrogen" in feces [1]	3.0	5.7	5.8	8.0
(d) Food nitrogen in feces (b—c) [1]	4.6	12.2	11.6	7.5
(e) Total nitrogen intake [1]	30.4	60.7	60.1	76.7
(f) Nitrogen absorbed (e—d) [1]	25.8	48.5	48.5	69.2
(g) Total urinary nitrogen [1]	29.0	31.6	31.8	21.7
(h) Endogenous nitrogen in urine [1]	11.4	11.4	11.9	13.6
(i) Food nitrogen in urine —(g—h) [1]	17.6	20.2	19.9	8.1
(j) Food nitrogen retained (f—i) [1]	8.2	28.3	28.6	61.1
(k) Biological value: $\frac{j}{f} \times 100$	31.8	58.4	58.9	88.3
(l) True digestibility coefficient: $\frac{f}{e} \times 100$	84.8	79.9	80.7	90.2
(m) Net protein value (a × k × l)	14.9	22.2	22.7	44.1

[1] Results expressed in milligrams of nitrogen per day. Values are average of 10 rats.

with the vitamins listed in table 1. All rations were kept in the refrigerator until needed. The trials lasted for 4 weeks.

The growth curves of the different groups of rats appear in figure 1. Examination of them shows that the ration containing *Torula utilis* as the sole source of protein gave the poorest growth performance, yet this same ration when sup-

[3] Vitamin test casein, General Biochemicals, Inc., Chagrin Falls, Ohio.

TABLE 5

Growth-promoting value of proteins in rations
(Results expressed on 4-week basis)

RATION	PROTEIN IN RATION BY ANALYSIS	NO. OF RATS PER GROUP	AVERAGE INITIAL WEIGHT	AVERAGE GAIN IN WEIGHT "a"	AVERAGE DIET CONSUMED	AVERAGE PROTEIN CONSUMED "b"	GROWTH-PROMOTING VALUE OF PROTEIN a/b
	%		gm	gm	gm	gm	
Torula yeast No. 1084	17.62	9	56.7	22.4	138.6	24.94	0.9
Bakers' yeast No. 51	17.69	9	54.8	43.3	176.2	31.71	1.4
Brewers' yeast No. 52	17.94	9	55.1	57.4	187.7	33.78	1.7
Purified casein	18.03	12	54.6	60.6	175.0	31.50	1.9
Torula yeast No. 1084 plus 0.5% DL-methionine	18.06	6	56.1	84.3	233.1	41.95	2.0

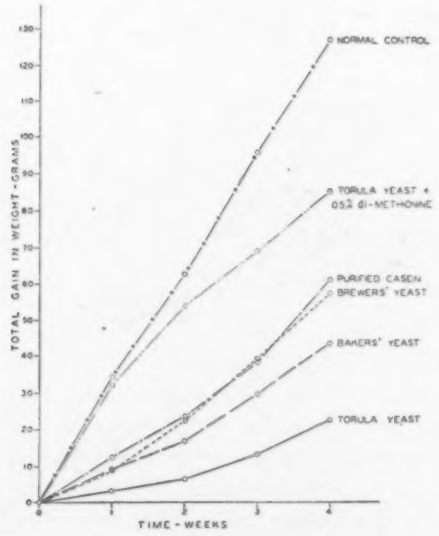

Fig. 1 Growth curves of groups of rats fed the different rations.

plemented with 0.5% DL-methionine gave the best performance of all.

Rations containing the two different strains of *S. cerevisiae* supported growth at somewhat different rates. The brewers' strain paralleled the growth performance of the ration containing casein, while bakers' strain had a poorer rate of growth though still much better than that of the *Torula* ration. Table 5 presents a summary of the details of this experiment, with calculations of the average gain in weight per gram of protein consumed.

The gain in weight of normal rats on our stock colony ration No. 1 (Goettsch, '46) for a similar 4-week period averaged 125 gm, a much superior gain to that obtained on any of the rations fed in this experiment.

DISCUSSION

The true digestibilities thus obtained were as follows: 84.8 for *Torula* yeast No. 1084; 90.2 for *Torula* yeast No. 1084 supplemented with methionine; 79.9 for Brewers' Yeast No. 52; and 80.7 for Bakers' Yeast No. 51.

Growth-promoting, biological, and net protein values of *Torula* yeast protein were decidedly lower than those demonstrated by the two strains of *S. cerevisiae.* As these three yeasts were prepared under identical conditions, it would seem that the lower nutritional value exhibited by *Torula utilis* under the conditions of this experiment was due to an inherent property of these pseudo-yeast proteins, and not to the environmental and nutritional factors that acted on the organism during its growth and development.

In a previous experiment (Goyco and Asenjo, '46) with a sample of *Torula utilis* No. 1084 prepared in England in a molasses-salts mash, the biological value reported was higher than the one obtained here — 48.8 as compared to 31.8 for the sample prepared in Puerto Rico in a similar, although not identical, mash. Even the former value of 48.8 was much lower than those biological values recorded by the present

authors in 1946 and by others (Sure and House, '49; Hughes and Hauge, '45), for different strains of *S. cerevisiae.*

In the present experiment the food intake was somewhat lower than that observed in the previously reported trial with samples of *Torula* manufactured in England (Goyco and Asenjo, '46). As a result of this lower food intake a negative nitrogen balance was induced in the animals, as can be seen in table 3. However, the rats consumed over 150 Cal. in non-protein constituents of the diet and not less than 500 mg of nitrogen per kilogram of body weight per day (compare Boas Fixsen, '30).

The great improvement noted in the performance of *Torula* protein on addition of 0.5% DL-methionine to the ration was remarkable. The recorded biological and growth-promoting values were almost three times as high as the ones for *Torula* protein alone, and are of an order exhibited only by the very best quality proteins. It is apparent that *Torula* yeast has an appreciable amount of its methionine in an unavailable form.[4]

These findings agree with those of Klose and Fevold ('45), who observed that the nutritional inadequacy of yeast protein could be corrected by the addition of methionine. The small amount of methionine added to the diet under discussion apparently stimulated the food intake of the experimental animals. There was an average intake of 2.5 gm of diet per day, without methionine; the addition of 0.5% methionine increased the intake to 6.3 gm per day.

Although the two strains of *S. cerevisiae,* Bakers' No. 51 and Brewers' No. 52, exhibited almost identical biological values (58.9 and 58.4, respectively), they differed significantly in growth-promoting values. Strain 51 had a growth-promoting value of 1.4, while 52 had a much higher one, 1.7. This difference between the two strains would indicate that the protein in the brewers' strain is more suitable for stimulating

[4] The three yeasts used were analyzed for methionine by the method of McCarthy and Sullivan ('41) using pepsin as the hydrolizing agent. As fed they contained respectively: yeast No. 1084 — 0.67%; yeast No. 51 — 0.91%, and yeast No. 52 — 0.69% methionine.

growth than that in bakers' yeast. Both, however, seem to function equally well for maintenance purposes.

SUMMARY

The nutritive value of the proteins of three different types of yeasts prepared under identical conditions was evaluated by means of the nitrogen balance and growth-promoting methods.

The *Saccharomyces cerevisiae,* Bakers' No. 51 and Brewers' No. 52, and *Torula utilis* No. 1084 were found, respectively, to be 80.7, 79.9, and 84.8% digestible; to possess biological values of 58.9, 58.4, and 31.8; to have net protein values of 22.7, 22.2, and 14.9; and to possess a protein efficiency of 1.4, 1.7, and 0.9.

With the exception of the coefficient of digestibility, the nutritive coefficients of *S. cerevisiae* strains were generally much higher than those of the *Torula.*

The protein of *Torula utilis* No. 1084, when supplemented by 0.5% methionine, gave the following nutritive coefficients: digestibility, 90.2; biological value, 88.3; net protein value, 44.1; and protein efficiency, 2.0.

The two strains of *S. cerevisiae,* Bakers' No. 51 and Brewers' No. 52, had similar nutritive coefficients with the exception of that for protein efficiency, which was higher in the case of strain 52.

LITERATURE CITED

BOAS, FIXSEN, M. A. 1930 The biological values of proteins. II. The biological value of purified caseinogen and the influence of vitamin B, upon the biological values, determined by the balance sheet method. Biochemical J., *24:* 1794.

GOETTSCH, M. 1946 Growth and reproduction in the rat on diets of rice and beans. Puerto Rico J. Pub. Health Trop. Med., *21:* 239.

GOYCO, J. A., AND C. F. ASENJO 1946 The net protein value of food yeast. J. Nutrition, *33:* 593.

HUGHES. C. W., AND S. M. HAUGE 1945 Nutritive value of distillers' dried solubles as a source of protein. Ibid., *30:* 245.

KLOSE, A. A., AND H. L. FEVOLD 1945 Nutritional value of yeast protein to the rat and the chick. Ibid., *29:* 421.

McCarthy, T. E., and M. X. Sullivan 1941 A new and highly specific test for methionine. J. Biol. Chem., *141:* 871.

Mitchell, H. H., and G. G. Carman 1924 The biological value for maintenance and growth of the proteins of whole wheat, eggs and pork. Ibid., *60:* 613.

Sure, B., and F. House 1949 Protein utilization of various dried food yeasts. Arch. Biochem., *20:* 55.

THE ESSENTIAL AMINO ACID CONTENT OF SEVERAL VEGETABLES [1]

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The general use of microbiological methods for the determination of amino acids has produced a considerable volume of published data on the amino acid contents of pure proteins, meat, eggs, other animal products, and cereal grains. However, very little data on the amino acid contents of vegetables are available. Stokes et al. ('45) give values for the essential amino acid content of carrots, peas, and potatoes. Whole dried corn has been investigated for its amino acid content by a number of workers (Horn et al., '46, '48; Schweigert, '47; Jones et al., '48) but very little if any data are available on the amino acid content of fresh sweet corn.

In this paper are reported the percentages of essential amino acids in broccoli, cauliflower, and several varieties of sweet corn and carrots. The consumption of these vegetables, especially carrots, has increased in the U. S. in the last few years (Clark et al., '47).

EXPERIMENTAL

Samples of each vegetable, or variety of vegetable, were collected at random, on the same day, from carefully controlled irrigated plots at the University of Arizona Vegetable Research Farm. Thus the different varieties of vegetable received the same fertilizer and irrigation treatment and

[1] Aided in part by a grant from the Williams-Waterman Fund for the combat of dietary diseases.

were subject to the same climatic variations. Two samples of broccoli of the Italian sprouting variety were assayed; one was collected at a different date than the other. Two varieties of cauliflower, Snowball and Snowdrift; 4 varieties of sweet corn, Yellow Hybrid, Kingcross, Tendergold and Seneca Chief; and 9 varieties of carrots, Imperator, Rubicon, Touchon, Tempe, Hutchinson, Morse's Bunching, Danver's Half Long, Red Core Chatenay and Scarlet Nantes were assayed. The vegetables were steam blanched for 5 min. and then dried overnight in a Stewart Warner dehydrator at 150°F. Moisture determinations were made on the dehydrated materials.

For assay of the essential amino acids other than tryptophan, accurately weighed quantities of the dried vegetables were refluxed 24 hours with 20% hydrochloric acid. The hydrolysates were evaporated on a hot plate to a syrupy consistency, the pH adjusted to 6.8–7.0, and the hydrolysates then diluted to proper volume and filtered.

For the assay of tryptophan, accurately weighed samples were autoclaved at 121°C. for 5 hours with 5 N sodium hydroxide (Krehl et al., '46). Fifty milligrams of cysteine were added at the beginning of the hydrolysis and again after one to two hours. As was found by Kuiken et al. ('47), this technique prevented the destruction of tryptophan. The final results were multiplied by two to correct for the racemization of tryptophan caused by the alkaline hydrolysis.

The hydrolysates were assayed for the essential amino acids almost exactly as indicated by Henderson and Snell ('48). Wasserman tubes (13 mm x 100 mm) were used instead of the 18 mm x 150 mm tubes specified by the above authors. For growing bacteria 10% filtered tomato juice was added to the media. For tryptophan both *Lactobacillus arabinosus 17-5* and *Streptococcus faecalis R* were used.

For all the amino acids [2] except isoleucine, values obtained are expressed on the basis of 100% activity for the L-isomer and 50% activity for the DL-isomer. For DL-isoleucine the

[2] All amino acids used for the standard curves were manufactured by Merck and Company.

values obtained are expressed on the basis of 39% of the activity of the L-isomer (Brickson et al., '48). Where both the L-form and the DL-form of the amino acid were available commercially, the responses of the assay organism to each were determined.

To check on the hydrolysis and microbiological procedure, recoveries were run. Amounts of amino acids approximately equal to those naturally present in the samples were added before hydrolysis. Also one sample of each vegetable was refluxed 40 hours with 20% HCl and the amino acid content

TABLE 1

Recovery of amino acids added before hydrolysis

AMINO ACID	BROCCOLI	CAULIFLOWER	CARROT	SWEET CORN
	%	%	%	%
L-Arginine	91	94	100	103
L-Histidine	96	95	95	100
DL-Isoleucine	102	100	97	108
L-Leucine	96	104	107	107
L-Lysine	110	95	92	..
DL-Methionine	103	109	97	96
DL-Phenylalanine	105	97	97	114
DL-Threonine	101	113	100	90
DL-Tryptophan	95	100	105	102
DL-Valine	108	113	93	113
Mean	101	102	98	104

of this hydrolysate was compared with that of the 24-hour hydrolysate to determine whether the latter period was sufficient to release each one of the amino acids completely.

RESULTS

The percentages of recovery of the amino acids added previous to hydrolysis are given in table 1. For all amino acids the recoveries are as good as can be expected using microbiological methods. The mean of the recoveries for sweet corn (104%) is slightly higher than for the other vegetables. It is possible that sweet corn contains substances, or

produces substances during hydrolysis, that slightly increase the response of microorganisms to several of the essential amino acids, especially to phenylalanine, leucine, and isoleucine.

Forty hours of hydrolysis did not liberate appreciably more of any amino acid than did 24 hours of hydrolysis, but did give 10% lower results for phenylalanine.

The response of *L. arabinosus* to DL-leucine, DL-phenylalanine, and DL-tryptophan, and of *L. mesenteroides* to DL-histidine and DL-lysine, was one-half as much as the response to the respective L-isomers. However, L-leucine, L-histidine and L-lysine were used in the standard curves.

In table 2 are given the percentages of essential amino acids in the vegetables, calculated both on the dry basis and on the basis of protein content ($N \times 6.25$). Referring to the work of Dunn ('47), and assuming that the amino acids are present in whole egg in optimum proportions, it follows that all these vegetable proteins are deficient in lysine and isoleucine. Cauliflower proteins compare fairly well with beef muscle proteins, being definitely deficient in three amino acids —isoleucine, leucine, and lysine—and possibly in two others, phenylalanine and arginine. According to Dunn ('47), beef muscle protein is not optimum in 5 of the essential amino acids. Broccoli is deficient in 8, being optimum only in arginine and methionine. Carrot protein is deficient in all the essential amino acids. Sweet corn is approximately optimum in histidine, leucine, phenylalanine and valine. A mixture of broccoli, cauliflower and sweet corn would afford proteins balanced in all the essential amino acids except lysine and isoleucine.

The variation in the percentages of amino acid in the proteins of the different varieties of corn and carrots is striking. Even though soil and climatic conditions were exactly the same for the different varieties tested there is no conclusive proof that one variety will be consistently better in any one amino acid than any other variety. It is well recognized that tests for nutritive value of different varieties of food crops

TABLE 2

Essential amino acid content of some vegetables

	BROCCOLI		CAULIFLOWER		CARROTS		SWEET CORN		WHOLE EGG (DUNN, '47)
NO. VARIETIES TESTED	1		2		9		4		
PROTEIN CONTENT	29.4 (25.3–33.5)		24.3 (22.6–25.9)		6–0 ± 0.24 [1] (5.3 ± 7.0)		16.1 ± 0.98 (14.3 ± 18.9)		
AMINO ACID %	Sample	Prot.	Sample	Prot.	Sample	Prot.	Sample	Prot.	Prot.
Methionine	0.70 (0.64–0.76)	2.4 (2.3–2.5)	0.63 (0.63–0.63)	2.6 (2.4–2.8)	0.057 ± 0.003 (0.039–0.074)	0.96 ± 0.07 (0.76–1.25)	0.26 ± 0.007 (0.24–0.27)	1.7 ± 0.11 (1.4–1.9)	3.1
Phenylalanine	0.97 (0.92–1.02)	3.3 (3.0–3.6)	0.88 (0.84–0.92)	3.7 (3.7–3.6)	0.25 ± 0.015 (0.18–0.34)	4.1 ± 0.24 (3.4–5.1)	0.98 ± 0.16 (0.73–1.41)	6.0 ± 0.59 (5.0–7.5)	5.4
Valine	1.14 (1.10–1.17)	3.9 (3.3–4.6)	1.54 (1.44–1.64)	6.4 (5.6–7.3)	0.34 ± 0.014 (0.27–0.42)	5.7 ± 0.24 (4.7–6.8)	1.07 ± 0.079 (0.89–1.26)	6.6 ± 0.11 (6.3–7.2)	7.1
Leucine	1.80 (1.60–1.99)	6.1 (5.9–6.3)	1.76 (1.66–1.85)	7.2 (7.1–7.4)	0.33 ± 0.018 (0.28–0.43)	5.6 ± 0.072 (4.6–6.4)	1.74 ± 0.091 (1.51–1.93)	10.8 ± 0.27 (10.2–11.3)	9.2
Isoleucine	1.05 (0.85–1.24)	3.6 (3.4–3.7)	1.05 (0.93–1.16)	4.4 (4.1–4.6)	0.26 ± 0.012 (0.23–0.29)	4.4 ± 0.14 (3.9–4.8)	0.61 ± 0.110 (0.51–0.68)	3.8 ± 0.33 (3.4–4.4)	6.7
Threonine	1.10 (0.99–1.21)	3.8 (3.6–3.9)	1.20 (1.16–1.23)	5.0 (4.8–5.1)	0.24 ± 0.005 (0.21–0.26)	4.0 ± 0.12 (3.4–4.5)	0.67 ± 0.071 (0.54–0.87)	4.1 ± 0.17 (3.9–4.6)	5.3
Lysine	1.54 (1.45–1.63)	5.3 (4.9–5.7)	1.35 (1.30–1.39)	5.6 (5.4–5.8)	0.18 ± 0.012 (0.13–0.22)	3.1 ± 0.19 (2.3–4.0)	0.61 ± 0.070 (0.56–0.71)	3.8 ± 0.07 (3.6–3.9)	7.3
Histidine	0.54	1.6	0.50 (0.44–0.57)	2.1 (2.0–2.2)	0.09 ± 0.000 (0.08–0.11)	1.5 ± 0.08 (1.2–1.9)	0.41 ± 0.020 (0.38–0.48)	2.6 ± 0.05 (2.5–2.7)	2.4
Arginine	1.71 (1.47–1.94)	5.8 (5.8–5.8)	1.18 (1.13–1.22)	4.9 (4.7–5.0)	0.21 ± 0.020 (0.10–0.30)	3.6 ± 0.39 (1.4–5.5)	0.79 ± 0.080 (0.69–0.99)	4.9 ± 0.48 (4.0–6.2)	5.7
Tryptophan	0.36 (0.32–0.39)	1.3 (1.2–1.3)	0.37 (0.34–0.40)	1.6 (1.3–1.8)	0.036 ± 0.0005 (0.029–0.043)	0.6 ± 0.07 (0.53–0.78)	0.081 ± 0.004 (0.075–0.090)	0.5 ± 0.00 (0.5–0.5)	1.6

[1] Standard deviation of the mean.

must be conducted over a number of years before any differences can be assumed as significant. However, our results indicate that the amino acid constituents of plant proteins may be affected by certain factors. This also is borne out by the work of Schweigert ('47), who reported considerable differences in the leucine content of the protein in two different samples of milo. The protein content of this author's samples varied much less than the protein content of either our corn or carrot samples.

SUMMARY

The content of essential amino acids was determined in broccoli, two varieties of cauliflower, 9 varieties of carrots, and 4 varieties of sweet corn. The protein of cauliflower as compared with whole egg protein is fairly well balanced in essential amino acids. It is definitely optimum in methionine, valine, threonine, histidine, tryptophan, and possibly arginine and phenylalanine. Sweet corn protein is approximately optimum in histidine, leucine, phenylalanine and valine, and broccoli protein in arginine and methionine. Carrot protein is deficient in all the essential amino acids.

The percentage of amino acids in the above plant proteins varied in the different samples. The variation is not attributed to difference in variety.

LITERATURE CITED

BRICKSON, W. L., L. M. HENDERSON, I. SOLHJELL AND C. A. ELVEHJEM 1948 Antagonism of amino acids in the growth of lactic acid bacteria. J. Biol. Chem., *176:* 517.

CLARK, F., B. FRIEND AND M. C. BURK 1947 Nutritive value of the per capita food supply. U. S. Dept. Agric. Misc. Pub. 616.

DUNN, M. S. 1947 Amino acids in food and analytical methods for their determination. Food Technology, *1:* 269.

HENDERSON, L. M., AND E. E. SNELL 1948 A uniform medium for determination of amino acids with various microorganisms. J. Biol. Chem., *172:* 15.

HORN, M. J., D. B. JONES AND A. E. BLUM 1946 Colorimetric determination of methionine in proteins and foods. Ibid., *166:* 313.

———— 1948 Microbiological determination of histidine in proteins and foods. Ibid., *172:* 149.

JONES, D. B., A. CALDWELL AND K. D. WIDNESS 1948 Comparative growth-promoting values of the proteins of cereal grains. J. Nutrition, *35:* 639.

KREHL, W. A., J. DE LA HUERGA AND C. A. ELVEHJEM 1946 Tryptophan studies. 1. Effect of niacin on the utilization of tryptophan. J. Biol. Chem., *164:* 551.

KUIKEN, K. A., C. M. LYMAN AND F. HALE 1947 Factors which influence the stability of tryptophan during hydrolysis of proteins in alkaline solution. Ibid., *171:* 551.

SCHWEIGERT, B. S. 1947 Amino acid content of feeds. 1. Leucine, valine, isoleucine and phenylalanine. J. Nutrition, *33:* 553.

STOKES, J. L., M. GUNNESS, I. M. DWYER AND M. C. CASWELL 1945 Microbiological methods for the determination of amino acids. II. A uniform assay for the ten essential amino acids. J. Biol. Chem., *160:* 35.

A PARADOXICAL RELATIONSHIP BETWEEN SERUM LEVEL AND LIVER CONTENT OF VITAMIN A[1]

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ONE FIGURE

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At the present time at least two views exist regarding the relationship between blood levels and the amount of vitamin A stored in the liver. Steigmann and Popper ('44) believe that if a sufficiently large number of cases is studied, some degree of parallelism may be observed between blood and liver levels. This has been attributed to the free vitamin A concentration in the liver (Glover, Goodwin and Morton, '47). Lewis, Bodansky, Falk and McGuire ('42), on the other hand, feel that blood levels of vitamin A are regulated by some means other than the amount stored in the liver. On reviewing the evidence in support of these two views it seemed probable that conditions such as age, sex, amount of supplementation and the degree of vitamin A depletion might determine which process regulates the relationship between vitamin A blood and liver levels.

The work of Lewis et al. ('42) was done on young male and female rats, three to 4 weeks old, that had been given supplements prior to depletion tests. It was therefore decided to examine this blood-liver relationship in older rats that had not received vitamin A supplements, and in similar rats under the influence of varying degrees of vitamin A depletion.

[1] This work was supported in part by grants from Merck and Co., Inc., Milbank Memorial Fund and National Vitamin Foundation, Inc.

METHODS AND PROCEDURES

Rats used were from a commercially supplied Wistar strain. The females weighed 125 to 150 gm and the males 175 to 225 gm at the beginning of the study.

Blood samples were taken by cardiac puncture and total livers removed from 51 female and 61 male rats; 27 males and 19 females were on normal diets [2] and the remainder were on a vitamin A-free diet [3] for periods ranging from 4 to 20 weeks.

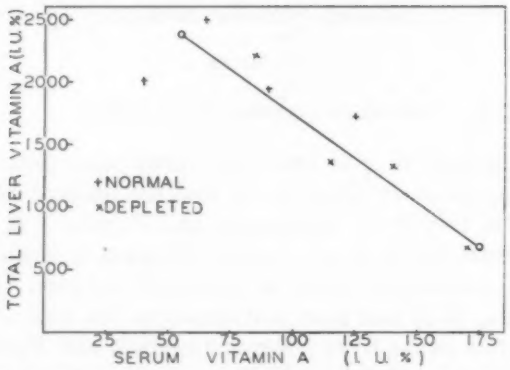

Fig. 1 The relationship between serum vitamin A and total liver content in normal and partially depleted female rats.

All animals were fasted 12 hours prior to taking the samples. Serum vitamin A was determined on duplicate samples by the method of Bessey et al. ('46). The livers were weighed, finely sectioned, thoroughly mixed and aliquots taken for the determination of vitamin A by the method of Oser et al. ('43).

RESULTS

A summary of the results is presented in figure 1 and tables 1 and 2. The points for the graph were determined by arranging the data into groups for which the interval for serum levels of vitamin A was 25 I.U./100 ml. The average serum

[2] Rats on a Purina Laboratory Chow diet.
[3] Rats on a vitamin A test diet, U.S.P. XII.

level in each group was plotted against the corresponding average value for the total vitamin A in the liver. In this figure the points were determined and plotted separately for normal and depleted females. The line of regression drawn between the points for normal and depleted female rats was determined by the method of least squares. A relation similar to the above for females was noted for normal male rats.

TABLE 1

Average weight, serum level and total liver content of vitamin A in normal and partially depleted rats

DIET	NO. OF RATS	WEIGHT		SERUM LEVEL			TOTAL LIVER LEVEL		
		Ave.	S.D.	Ave.	S.D.	Range	Ave.	S.D.	Range
Females		*gm*		*I.U. %*			*I.U.*		
Normal	27	140	± 15	70	± 37	(55–125)	2,226	± 705	(1,019–3,280)
Vit. A-free, 4 wks.	6	160	± 18	135	± 24	(110–175)	1,161	± 172	(850–1,360)
Vit. A-free, 8 wks.	6	180	± 12	115	± 26	(55–150)	1,368	± 681	(715–1,960)
Vit. A-free, 16 wks.	6	190	± 11	115	± 34	(80–165)	1,436	± 946	(141–3,020)
Vit. A-free, 20 wks.	6	190	± 10	140	± 26	(125–175)	1,073	± 511	(600–2,130)
Males									
Normal	19	200	± 24	125	± 49	(55–175)	3,021	± 1727	(1,517–6,230)
Vit. A-free, 4 wks.	10	250	± 25	135	± 46	(25–200)	362	± 156	(100–600)
Vit. A-free, 8 wks.	20	279	± 26	95	± 52	(20–165)	174	± 126	(39–499)
Vit. A-free, 12 wks.	12	304	± 23	65	± 45	(0–155)	61	± 26	(27–106)

The relationship between the serum vitamin A level and liver content of the partially depleted male rats is presented in table 2. These rats were divided into two groups on the basis of their liver content of vitamin A and not according to the time on the vitamin A-deficient diet. In group I were placed those rats whose livers contained only apparent vitamin A; the remaining depleted male rats, whose total liver vitamin A content ranged between 103 and 499 I.U., were placed in group 2.

TABLE 2

The serum level and liver content of two groups of partially depleted male rats

	NO. RATS	TOTAL LIVER CONTENT		SERUM LEVEL	
		Ave.	Range	Ave.	S.D.
		I.U.		*I.U.*	
Group I	19	63 [1]	36–95	75	48
Group II	23	277	103–499	120	44
Serum level difference (group II — group I)				45 [2]	

[1] This value should be labelled "apparent vitamin A," since the color developed by adding the Carr-Price reagent to the extracts from these livers did not produce a characteristic color for vitamin A. A similar observation has been described by Mattson et al. ('47).

[2] The standard error of the difference between the mean serum levels as determined by the formula $\sigma_D = \sqrt{\frac{\sigma_1^2}{N_1} + \frac{\sigma_2^2}{N_2}}$ (Arkin and Colton, '46) was found to be 14 I.U., thus indicating the statistical significance of the difference between the mean serum levels for groups I and II.

It is evident from an examination of these data that an inverse relationship exists between the blood and liver levels of vitamin A in normal male and female rats within the ranges tested. It may also be noted that this inverse relationship holds true in the case of the vitamin A-depleted female animals. The coefficient of correlation [4] for the line of regression as drawn in figure 1 was found to be —0.95, indicating that the relationship is significant.

In the male rats the depletion was more severe than in the females, as is indicated by the lower vitamin A content of the

[4] $r = \sqrt{1 - \frac{\sigma y^2}{y^2}}$ (Arkin and Colton, '46).

liver (see tables 1 and 2). Here a direct relationship was found between the blood and liver levels; that is, as the liver content decreased the serum level also decreased.

From table 1 it will be noted that the average serum vitamin A levels were lower and the liver content higher in the normal female group of rats than in those subjected to the stress of depletion. In the males, however, the serum level and liver content were both lower in the depleted group than in the normal group.

It may be noted that there was a sharp decline in the average amount of vitamin A in the livers of rats after they were on a deficient diet for 4 weeks. The males were more severely affected than the females. Some female rats had as high as 3,000 I.U./total liver after being on a vitamin A-free diet for 20 weeks, whereas in the male group (12 rats), after a similar diet for 12 weeks, all except one had a liver content of vitamin A of less than 100 I.U. The inverse relationship between blood and liver levels held for the former but not for the latter case.

The degree of stress placed on these animals was not sufficient to produce any of the usually accepted physical signs of vitamin A deficiency. The male rats gained weight throughout the test period, whereas the females, after 12 weeks of depletion, did not show an increase in weight but remained constant up to 20 weeks.

DISCUSSION

The evidence presented in figure 1 supports the hypothesis that there is a regulatory mechanism controlling the blood vitamin A level of rats which, under the conditions of this experiment, operates to give an inverse relationship between the blood level and liver content of vitamin A. This regulatory process was found to function in both normal male and female rats and also in those females subjected to vitamin A depletion. It is obvious, however, that the range of this relationship is limited and that it would not apply under conditions of excessive supplementation or extreme depletion. This

latter limitation is shown by the group of male rats on the vitamin A-free diet, in which it will be noted that a low liver content is associated with a low serum level. This change in relationship may be attributed to a breakdown of the regulatory mechanism by the depleted state of the animal. It is of course possible that certain sexual factors may also affect this process.

Sex differences in the rate of loss of liver stores have been previously summarized by Brenner et al. ('42) as favoring the females. This view is further substantiated by the present findings; namely, that in general the male rats were more severely depleted of their vitamin A liver stores in a period of 4 weeks than were the females over a 20-week period.

If low liver content and serum levels of vitamin A are an indication of a deficiency state, it is difficult to account for the continued increase in weight of all male rats, especially in those whose serum levels were zero and whose livers contained less than 100 I.U. of apparent vitamin A. It seems to the author that either vitamin A is not as important a factor in the growth and development of older rats as it is for younger rats, or that a measure of the liver content and serum level is not adequate for determining a deficiency state.

This study confirms the rapid loss of the vitamin A liver depots in the first 4 weeks as noted by Popper and Brenner ('42) and Davies and Moore ('35). The evidence contained in histological studies by Popper and Brenner ('42) and the more recent work of Glover and Morton ('48) suggests that this early and great loss may involve a specific labile type of vitamin A, stored in the hepatic cells as a protein complex, which in turn is readily available for liberation into the blood stream. The more gradually depleted form may represent that vitamin A stored in the Kupffer cells.

It is impossible, with the evidence at hand, to present a satisfactory explanation for the two relationships found to exist between liver content and serum level of vitamin A. It is suggested on the basis of the inverse relationship that perhaps tissue demand is a more potent factor governing serum

blood levels than is liver depot content. Various factors such as age, sex, disease and amount of vitamin A stored undoubtedly affect this relationship.

In conclusion it may be stated that the evidence gained from this study plus the observations described by other workers (see above) support the view that it is difficult to assess the body's need or its reserve of vitamin A on the basis of blood level per se. In light of these findings it seems evident that additional investigation must be made in order to determine whether these factors affect the serum vitamin A level in man.

SUMMARY

An examination was made of the relationship between serum level and liver content of vitamin A in normal rats and those under the influence of varying degrees of vitamin A depletion. The following points were observed:

(1) An inverse relationship existed between blood and liver levels of vitamin A in normal male and female rats and those depleted female animals whose liver content ranged as low as 600 I.U./total liver.

(2) When the total liver content fell below 600 I.U. in the males there was a parallelism between blood and liver levels.

LITERATURE CITED

ARKIN, H., AND R. R. COLTON 1946 An Outline of Statistical Methods. Barnes and Noble, Inc., New York.

BESSEY, O. A., O. H. LOWRY, M. J. BROCK AND J. A. LOPEZ 1946 The determination of vitamin A and carotene in small quantities of blood serum. J. Biol. Chem., *166:* 177.

BRENNER, S., M. C. H. BROOKES AND L. J. ROBERTS 1942 The relation of liver stores to the occurrence of early signs of vitamin A deficiency in the white rat. J. Nutrition, *23:* 459.

DAVIES, A. W., AND T. MOORE 1935 Vitamin A and carotene: elimination of vitamin A from livers of rats previously given massive doses of vitamin A concentrates. Biochem. J., *29:* 147.

GLOVER, J., T. W. GOODWIN AND R. A. MORTON 1947 The relationship between blood vitamin A levels and liver stores in rats. Ibid., *41:* 97.

GLOVER, J., AND R. A. MORTON 1948 The administration, storage and metabolism of vitamin A. Ibid., *43* (XII, Proceedings of Biochemical Society).

LEWIS, J. M., O. BODANSKY, K. G. FALK AND G. MCGUIRE 1942 Vitamin A requirements in the rat. The relation of vitamin A intake to growth and to concentration of vitamin A in the blood plasma, liver and retina. J. Nutrition, *23:* 351.

MATTSON, F. H., J. W. MEHL AND H. J. DEUEL, JR. 1947 The site of conversion of carotene to vitamin A in the rat. Arch. Biochem., *15:* 65.

OSER, B. L., D. MELNICK AND M. PADER 1943 Estimation of vitamin A in food products. Ind. Eng. Chem., Anal. Ed., *15:* 724.

POPPER, H., AND S. BRENNER 1942 The fate of excess vitamin A stores during depletion. J. Nutrition, *23:* 431.

STEIGMANN, F., AND H. POPPER 1944 The influence of large doses of vitamin A upon the plasma vitamin A levels. Am. J. Med. Sci., *207:* 468.

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